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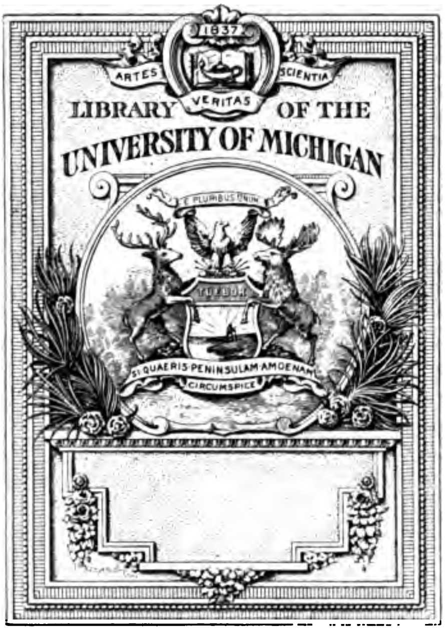
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BRYN MAWR COLLEGE MONOGRAPHS

REPRINT SERIES, Vol. VI.

CONTRIBUTIONS FROM THE BIOLOGICAL LABORATORY

BRYN MAWR, PENNA., U. S. A.
July, 1906

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Germ-Layers and Regeneration.

By

T. H. Morgan.

Eingegangen am 1. Februar 1904.

The principal result that Miss MARGARET REED has obtained from her study of the regeneration of the leg of the crayfish and hermit crab has interesting theoretical bearings to which I wish to refer briefly here, reserving a fuller discussion for a time when further observations of this kind have been made.

One of the most familiar facts of regeneration is that the proliferation to form the new tissues takes place from those parts that have been directly involved in the injury. In studying the process of autotomy in crabs and crayfishes I had become familiar with the fact that when the leg is thrown off at the breaking-joint none of the muscles are injured, since none cross this level of the leg. The question at once suggested itself as to whence come the cells that produce the muscles of the new leg. At my suggestion Miss REED has worked out this problem; and has determined that the cells that produce the new muscles are proliferated by the ectoderm of the leg itself. This result at once suggests a comparison with the mode of development of the muscles of the leg of the embryo.

From the researches of REICHENBACH on the crayfish, of HERRICK on *Alpheus* and on the lobster, and of Bumpus on the lobster, it appears that the mesoderm arises in large part, if not entirely, from or near the superficial plate of the early blastoderm that is subsequently invaginated to produce the archenteron. Hence, according to the usual criterion, the mesoderm is endodermal in origin. This distinction is perhaps largely formal, yet it is that usually accepted by those who refer all the organs back to ecto-, meso-, and endoderm. Whether or not some of the ectodermal cells in front of the endodermal plate do not also take part in the proliferation may still remain an open question. In any case it is clear that the mesoderm that goes to form the musculature of the limbs is supposed to arise in the embryo from the mesoderm proliferated at or around the region of the blastopore, and not from the ectoderm of the limb itself. It is evident therefore that the mesoderm to produce the muscles of the regenerated limb has a different origin from that which produces the muscles of the limbs of the embryo. It would also appear, according to the usual view, that the muscles arise in the two cases from different germ-layers; but this distinction does not appear to me as important as that of the localization of the material from which the mesoderm arises in the two cases.

In recent years a few other instances have been recorded in which a regenerated structure arises from a different region of the body from that from which it takes origin in the embryonic development. The most interesting case in this connection is that of the fresh water oligochaete worms. It has been shown by several recent writers that the cells that go to form the mesoderm of the new head and tail of these worms come from the ectoderm, and not from the old muscles, or at least not to any extent. Here also we appear to meet with a discrepency between the regenerative and the ontogenetic development, although, as I have pointed out elsewhere [1]), the early origin of the mesoderm in the annelids from the primary mesoblast makes it difficult to determine whether this layer is ecto- or endodermal, or whether, in fact, it does not have an origin that is quite independent of the two »primary germ-layers«. However this may be, the fact that concerns us here is sufficiently evident; namely, that although at the very beginning of the development the parts of the egg that are to give rise to ecto-, meso-, and endodermal structures are separated; yet in regeneration the ectodermal cells give rise to mesodermal structures, and this, too, despite the fact hatt they have been presumably functional ectodermal cells.

A few other cases in which the germ-layers are interchanged, as it were, are also known. It has been determined in the anemone, *Sagartia*, that the lining of the regenerated oesophagus is endodermal, while it is a characteristic of the entire group of *Scyphozoa* that the lining of the oesophagus is ectodermal. Here again we find one »layer« supplanting the other in the regenerative development, and in this case it is one of the »primary germ-layers« themselves that takes the place of the other.

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[1]) »Regeneration.« p. 212.

In the fresh water annelids, again, we find that while the lining of the pharynx of the individual that develops from the egg is ectodermal, it is endodermal in the regenerated head. A similar interchange takes place in the earthworm.

The case that has attracted the most attention in recent years is that of the regeneration of the lens in the salamander. In the embryo the lens develops, as in all other vertebrates, from the ectoderm of the side of the head. In regeneration it develops from the edge of the iris. In this case, it is true, the lens arises from the same »germ-layer« both in the regenerative and in the embryonic development, so that in this respect the result is less striking than in the other cases that I have referred to above, yet this distinction is, I think, of only subordinate value.

It is not my intention to enter here into the more problematical questions that arise in connection with the mode of regeneration of the mesoderm of the leg of the crayfish. Here, as in the other cases, we may easily run afoul of teleological questions. WOLFF, especially, has often referred to these in connection with his experiments on the regeneration of the lens. There is one point of difference between the case of the crayfish and that of the salamander that should not pass unnoticed; namely, that while in the crayfish the uninjured muscles do not proliferate cells to produce the new structure, in the salamander, on the other hand, the uninjured iris does produce the new cells.

Another point of interest is that the cells that produce the muscles of the new leg of the crayfish do not arise from the cut ends of the ectoderm, but appear only after the ectoderm has closed over the wounded surface. It is, therefore, from an intact ectodermal surface that the new cells originate. It may be a point of some significance in this connection, although I do not believe we are yet in a position to guage the real value of the phenomenon, that while the process of closing over the relatively large exposed surface is taking place there is no karyokinetic division of the cells, but as soon as the proliferation, that produces the cells that give rise to the nerve and to the new muscles, begins, active processes of mitotic division can be seen.

The questions concerning the stimulus that causes the ectoderm cells to proliferate inward, and the more profound question as to the origin of the power to regenerate one set of organs from a region of the body that does not produce cells of this sort during the embryonic development; — these questions we can not, I think, profitably discuss at present.

Summary.

In the regeneration of the leg of the crayfish the new muscles arise from the ectoderm, as shown by Reed; in the embrionic development the muscles are generally supposed to come from the region of the blastopore and are said to be derived from the endoderm. Thus the same structure arises from different germ-layers in the two cases. Analogous results have been obtained by other investigators in other forms. The value of the germ-layer-hypothesis appears to have less significance in the light of these facts, and the need of a different conception to account for the potentialities of the cells of the body becomes evident. The more important problems at present are to discover how certain cells retain in latent form some of the properties possessed at first by the whole egg-cell, and how other cells lose some of the properties, or, what amounts to nearly the same thing, are unable to bring them to development. Equally important is the problem of regulation of the factors that arouse in certain cells a response that is purposeful. We have at present no satisfactory solution for these questions.

Zusammenfassung.

Bei der Regeneration des Krebsbeins entspringen die neuen Muskeln vom Ektoderm, wie Reed zeigte; bei der Embryonalentwicklung nimmt man allgemein an, daß die Muskeln von der Gegend des Blastoporus herkommen und leitet sie vom Entoderm ab. Somit stammt dieselbe Struktur in den beiden Fällen von verschiedenen Keimblättern. Analoge Resultate haben andre Forscher bei andern Arten erhalten. Der Wert der Keimblättertheorie scheint im Lichte dieser Tatsachen zu verlieren und es erscheint eine Neuformulierung derselben, welche den Potenzen der Körperzellen Rechnung trägt, mit Evidenz als notwendig. Das gegenwärtig wichtigste Problem bildet die Ermittlung, wie gewisse Zellen in latenter Form manche Eigentümlichkeiten zurückbehalten, welche zuerst die ganze Eizelle besaß, und wie andre Zellen einige dieser Eigentümlichkeiten verlieren, oder was ziemlich auf dasselbe herauskommt, wie sie unfähig werden, dieselben zur Entwicklung zu bringen. Ebenso wichtig ist die Frage nach der Regulation der Faktoren, welche gewisse Zellen zu einer zweckmäßigen Reaktion anregen. Wir besitzen gegenwärtig keine befriedigende Lösung dieser Fragen.

The Relation Between Normal and Abnormal Development of the Embryo of the Frog: VI. As Determined by Incomplete Injury to One of the First Two Blastomeres.

By

T. H. Morgan.

With Plates XVII and XVIII and 7 figures in text.

Eingegangen am 16. Dezember 1904.

The conclusions that O. HERTWIG reached in 1893[1] and which he appears still to hold[2], in regard to the results of injury to one of the first two blastomeres of the frogs egg (ROUX's experiment), are in one fundamental respect so entirely different from the point of view that I have maintained in this series of papers dealing with the relation between normal and abnormal development that a more detailed examination of the problem is, I believe, desirable. I do not refer so much to the question as to whether half embryos can be obtained, as ROUX claimed, by injuring one of the first two blastomeres, for, recent experiments have shown that HERTWIG was mistaken in asserting that this does not occur, but I refer rather to HERTWIG's contention that the injured part of the egg assumes the same relation to the uninjured part that exists in other vertebrate embryos having a larger amount of yolk. This question, and the secondary questions that are involved in it, will be the main topic discussed in the following pages. It will be also necessary to consider again the question of ROUX's postgeneration in the light of some new observations.

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[1] Über den Wert der ersten Furchungszellen für die Organbildung des Embryo. Arch. f. mikr. Anat. XLII. 1893.

[2] Handbuch der vergleichenden und experimentellen Entwickelungslehre. XIV and XV. 1903.

A small series of experiments were made in order to find out more precisely what the result of incomplete injury really effects. By incomplete injury, I mean, injury with a hot needle that is insufficient to kill the blastomere. It is true that this same result is obtained in most cases in which ROUX's experiment is attempted, for, here also, as ROUX himself, HERTWIG, C. ZIEGLER, MORGAN, and MORGAN and TORELLE have shown the nucleus is left intact in a very large proportion of cases. Nevertheless by deliberately carrying out the experiment in such a way that no attempt is made to entirely destroy the injured blastomere, the side of the question that I wished to examine is better reached. I shall, however, also draw on other results that I have obtained with a hotter needle.

In my experiments a rather small needle was used, which was heated not quite to a red heat, and then, without haste, was thrust into the upper black part of one of the first two cells of an egg from which the outer membranes had been largely removed. The needle was not only somewhat cooled by the air before the puncture was made, but also by the remaining parts of the jelly. In addition the protoplasm also cooled the needle, so that by the time the nuclear region was reached the needle had, no doubt, become ineffective, and this also occurs, as has been said, in most cases where the experiment is carried out by ROUX's method.

The following six series of experiments cover the main points.

In the first series the eggs were stuck in the late two cell-stage. Two eggs produced normal embryos, five did not go further than a late segmentation stage, three formed a circular blastopore at or near the equator of the egg, but the parts of the embryo were not developed. Five other embryos show the following conditions. One is a half-embryo with the injured side cellulated, but without any indications of the formation of organs. The second embryo is a whole embryo with a posterior yolk-plug, due to the lack of development of the posterior lip of the blastopore. The third embryo is a whole embryo with much of the yolk portion of the egg vacuolated; the dorsal part of the embryo occupies almost the entire upper part of the egg. A longitudinal section of this embryo is drawn in Plate XVIII Fig. VIII. The fourth embryo has a whole anterior end (reduced in size) with the condition of spina bifida behind the head, but with the sides very little developed. The fifth embryo is apparently a whole embryo with one side more abnormal than the other.

In the second series all five eggs were preserved at the beginning of the gastrula-stage, which appeared nearly normal except for a small exovate at one side. Three of these are represented in Plate XVII Figs. 1, 2 and 3, and will be described later.

In the third series, stuck in the late two-celled stage, two embryos were nearly normal, showing the beginning of a symmetrical dorsal lip of a blastopore; the exovate lying above and to one side. One egg had lost so much substance that it was not more than half-size, yet was symmetrically developed.

In the fourth series five normal embryos with closed blastopore (i. e. with the yolk-plug withdrawn) were present; two others had a large exposure of yolk in the yolk hemisphere, and the medullary folds lay obliquely on the dark part as shown in Figs. 6 and 8.

In the fifth series thirteen embryos were normal, or nearly so, having the medullary folds well developed. In most cases a hernia-like protrusion, covered by dark ectoderm cells, was present on one side. Twelve embryos showed a retarded gastrula with a large yolk plug. The anterior end was usually present, lying straight forward or somewhat obliquely. Fourteen eggs were still in segmentation stages, and were more or less abnormal.

In the sixth series the eggs were stuck at the beginning of the two-cell stage. Forty-five embryos, apparently normal, were obtained. The following abnormal forms were also present: one half embryo with most of the injured half not cellulated; one anterior end of an embryo, reduced in size, extending to the equator of the egg; and one embryo lying obliquely along one side of the yolk.

Summary. The large percentage of normal embryos in these series indicates that early recovery more or less complete may take place after sticking the egg. A fair proportion of eggs were delayed in their early development, in others the embryo met with difficulty in growing over the yolk, or more accurately in withdrawing the yolk mass into the interior of the egg. The embryos that lie obliquely on the black hemisphere are similar to those described by Hertwig, and will be more fully considered below. Also those embryos that have only the anterior end present call for special treatment; and will be dealt with later.

From the preceding material the embryos represented in Plate XVII were picked out, drawn, and cut into sections. They will serve to show the great variety of forms that may result from the operation. Figure 1 is a ventral view of an embryo at the beginning of the gastrula-stage. A small protuberance on the side of the egg opposite the dorsal lip is present. A longitudinal section through this egg is represent in Fig. I Plate XVIII. The whole egg is cellulated excepting a small region near the protuberance. The region has not, it appears, been an obstacle sufficient be prevent nuclei from passing into the yolk parts of the injured side.

Fig. 2 is also an early gastrula-stage. The longitudinal[1] section, Fig. II, shows that only one half of the egg is cellulated, although the roof of the entire segmentation cavity is formed of cells, some of which must have come from the injured half. The injury to this half has, however, prevented, very largely, the down-wandering of nuclei into the yolk portions of the egg.

Fig. 3 represents another gastrula-stage in which the injured region extends down to the edge of the blastopore. Which portion of the edge represents the dorsal lip is not entirely clear, but it seems to lie to one side of the protuberance. A section of this egg, Fig. III, shows that the entire egg is cellulated, and that the protuberance lies just above the equator of the egg.

These three eggs show that the results of the injury to one of the blastomeres may appear in regions of the embryo which seem to be removed from the part actually stuck. If, however, as must often occur, the needle pierces the egg not exactly in the middle region of the top, but more towards one side, — anterior or posterior as the case may be — the effects will be those which actually appear. Moreover, when the needle is withdrawn, some of the substance of the egg often protrudes, and this may come more from the anterior or posterior side than from the top of the blastomere, and involve lower regions of the egg than that actually pierced.

Fig. 4 is a whole embryo of reduced size. A large exovate was formed, which was found in the hardened egg to be completely separated from the embryo. It consisted almost entirely of yolk, as shown in Fig. IV. On one side a well marked and characteristic invagination (or rather opening out of the yolk cells) is present, and represents the development of a part of the archenteron. This is all the more interesting as the exovate is largely, if not entirely, a part of the yolk region of the egg. It may, however, have been formed during the gastrula stage when it was still a part of the embryo.

Fig. 5 represents the anterior end of an embryo, extending far forward over the black hemisphere. One side is somewhat injured, as well as the ventral and posterior regions.

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[1] The section is probably also quite oblique.

Fig. 6 represents the anterior region of a whole embryo lying obliquely on the black part of the egg. The left side of the egg and the posterior yolk are injured.

Fig. 7 shows all that remained of an egg after a large amount of its substance was lost. In volume it is not more than half of an egg, yet it shows indications of gastrulating all round one side. A section of this egg is drawn in Fig. V. The outer surface is covered by ectoderm, beneath which is a layer of smaller cells. The interior is filled with loosely aggregated yolk cells, which come to the surface at one point. A distinct archenteron is present along the blastopore-rim.

Fig. 8 represents the dorsal region of a whole embryo lying more to one side, than to the other, on the dark part of the egg. The right side and the posterior parts of the egg are injured.

Fig. 9 is a half-embryo, or somewhat more than half in the anterior regions. The sections were so oblique that they add little information to what the surface shows.

Fig. 10 represents an embryo (best understood in longitudinal section in Fig. VI), that occupies almost the entire black portion of the egg. The injured part protrudes behind and to one side.

Fig. 11 shows a whole embryo, reduced in size, with a defective posterior lip. The exovate, which became detached when the egg-membranes were removed, showed when cut into sections, Fig. VII, a differentiation into the three germ-layers with a slight inturning at one end, which probably represents the posterior lip of the blastopore that is missing in the embryo. This exovate also may have been attached to the embryo and only later constricted off.

Fig. 12 represents the anterior end of an embryo lying obliquely on the dark part of the egg. The medullary plate is indicated, but the medullary folds have not begun to lift up.

The general interpretation of these embryo will follow from the account given in the next pages.

Interpretation of Results.

The kind of segmentation after partial injury to one blastomere. If the segmentation of an injured egg be watched it will be seen that, as a rule, the punctured cell segments, but more irregularly and often later than the uninjured half. Not infrequently the segmentation is confined at first to the upper, dark hemisphere, and the nucleosation and cell-formation in the yolk hemisphere may be delayed. The cellulated upper portions of the injured part are from the beginning coextensive with the cellulated portions derived from the other blastomere. Sections show that a segmentation cavity appears in the top of the egg, although on the injured side it is often less extensively developed than on the uninjured side. The condition of the egg at the end of the cleavage period will depend on which regions have been injured and which have become nucleated. If the injury involves only the outer side of the top of the injured blastomere, the egg will appear as in Diagram *A* 1. In this case

Diagram *A*.

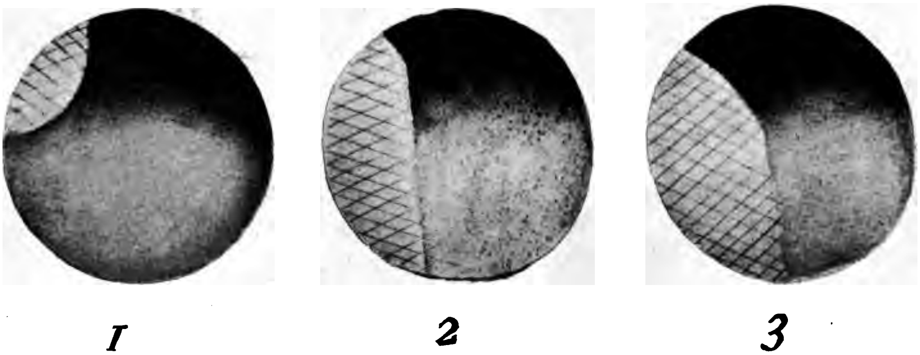

Fig. 1. Outer side only of blastomere injured. Fig. 2. Outer side of both top and bottom of blastomere injured. Fig. 3. All of blastomere except top injured.

nuclei have also passed down into the yolk portion and it has become divided into cells. If, however, the injury prevents or delays the passage of nuclei into the lower regions of the outer side of the blastomere the egg may appear as in Diagram *A* 2. Should the yolk entirely fail to receive nuclei the result will be like that indicated in Diagram *A* 3. According the which of these results follow the operation, the succeeding stage, during which gastrulation takes place, will be correspondingly affected.

Location of the dorsal lip. It appears that the dorsal lip of the blastopore is located approximately in the same part of the injured egg that it occupies in the normal egg[1]). It would appear, therefore, as shown in diagrams *B* 1 and *C* 1. It lies on the same side of the egg as that on which the gray crescent lay, and, as a rule, coincides approximately with the plane of the first division, although it may deviate to a greater or less extent from this plane — not however, so far as known, because of the injury to the egg, but because of an earlier relation already established. In other words, there is nothing in the results to indicate that the location of the dorsal lip shifts to a new position, as Hertwig implies, unless an early shifting in the protoplasm takes place soon after the operation, in consequence of a change in position of the egg in respect to gravity. If the latter should occur the material of the dorsal lip may be carried into a new position, as in Schultze's experiment, thus tending towards a new form of development, namely, a whole embryo of half size. This point will be discussed more fully later, but meanwhile it is simpler to deal with those cases in which the contents of the blastomeres retain after the operation their natural position, i. e. they

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[1]) An exception must be made in those cases in which the segmentation cavity fails to develop, when the blastopore appears higher up on the egg as explained in preceeding papers of this series.

Diagram *B*.

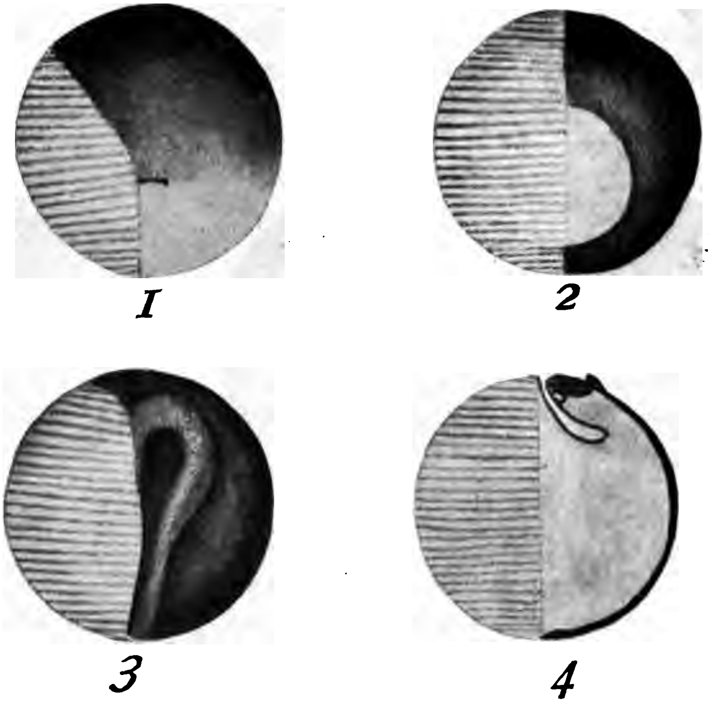

Fig. 1. Gastrula stage of Diagram *A* Fig. 3. Fig. 2. Later stage, seen more from lower hemisphere. Fig. 3. Half-embryo. Fig. 4. Cross-section of same.

stand »vertically« in regard to gravity. Furthermore in the eggs of this species of frog, the shifting of the contents of the first blastomeres appears to take place less easily than in some other forms.

Advance of the dorsal lip of the blastopore and the formation of the lateral lips. The effects of the injury become more apparent when the dorsal lip begins to advance over the lower hemisphere, and the lateral lips begin to appear. As the lateral lips develop around the sides, the lip of one side meets with the injured region and its progress is stopped. The same fact may be expressed more accurately by saying that the lateral lip fails to develop in the injured part, so that the blastopore remains incomplete on that side.

The extent to which the lateral lip is formed in an egg injured as shown in Diagram *A* 1 will depend to some extent on how much of the embryo-forming material has been injured near the top of the punctured blastomere. To a certain extent, no doubt, this loss may be made good by the rest of the embryo-forming material at the sides of the injured region. Nevertheless defects would be looked for in the middle part of the half of the embryo on the injured side.

When the injured region extends to the middle line as in Diagram *B* 1, the lateral lip on the uninjured half of the egg is completed, but practically none is present on the injured side. As the blastopore closes the dorsal lip may extend for some distance backwards, and the lateral lip of the uninjured side advance to the line of demarcation between the injured and the uninjured region, as shown in Diagram *B* 3. When, on the other hand, the injured region is less extensive and some of the lateral lip is formed on the injured side, Diagram *C* 1, 2, the backgrowth of the dorsal lip on that side may be more complete as shown in Diagram *C* 3. The extent to which the anterior end of the embryo forms on the injured side depends on the extent of overgrowth of the blastopore of that side, Diagram *C* 3, 4.

Formation of the Embryo. In the case where the injury is less extensive, and the region overgrown by the dorsal lip is greater, the embryo appears as represented in Diagram *C* 3. It appears to lie excentrically on the dark part of the egg, and extends along the border of the injured region. The apparently oblique position of the medullary folds is due in such cases to the embryo being more complete on one side than on the other. As explained above this is the necessary outcome of the relation of position of the dorsal lip to the injured part. Since the material that is carried backwards on the injured side is less and less in amount the farther back the region, this side of the embryo is correspondingly deficient in material the further back the region lies.

In the other case, where the injury extends to the middle line in the region below the equator of the egg, a half embryo is formed as shown in Diagram *B* 3. In its anterior half this embryo is usually more than a half-structure, because, in part, there is more than half of the material present in front of the dorsal lip out of which the head of the embryo develops, but also, because, even in those cases when one half of the egg has been entirely killed or injured, the head end is more nearly a whole structure from the beginning, as Roux [1]) and all subsequent workers have observed. The difference

Diagram *C.*

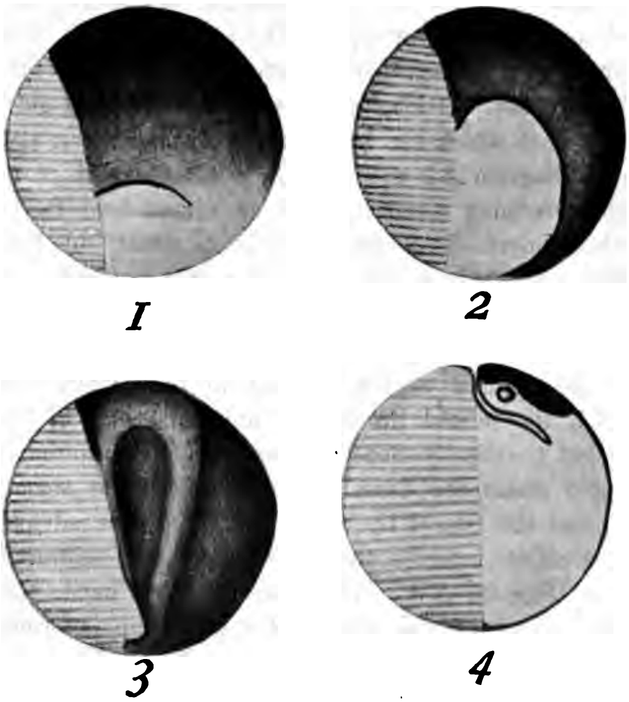

Fig. 1. Gastrula stage of egg in which the outer and lower region have been injured and only the top and inner portions of this blastomere have segmented. Fig. 2. Later stage of same, seen more from ventral view. Fig. 3. Embryo more than half. Fig. 4. Cross-section of same.

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[1]) With Roux's interpretation, viz.: that a half head first forms, which then postgenerates its missing half, I can not agree.

between the formation of the head region and of the more posterior regions in the normal embryo gives a clue to the difference that appears in the half-development, for, the head end develops from material that has always had a single origin, while the middle and posterior regions of the embryo come from the sides of the blastopore, and have had, therefore, a double origin. In the half embryo this relation appears to determine that the head region, when it differentiates out of the undivided material in front of the first position of the dorsal lip develops more nearly symmetrically, and forms a whole structure, while in the more posterior regions the conditions along the lateral lip of the blastopore of the uninjured side remain the same as in the normal embryo, and a half structure is produced.

There is another result that is met with not uncommonly in these experiments, namely, that in which only the anterior end of an embryo develops, and a ring-like blastopore extends around, or just below the equator of the egg. This result is due in most cases to different conditions from those just described. It may be said, in general, that the yolk hemisphere fails to be drawn in under the edge of the blastopore, but the result is primarily due to conditions that can be traced further back in the development, namely, to the failure of the embryo-forming substance to become transferred downwards, and this in turn is probably due, as I have suggested elsewhere¹) to the failure of the roof of the segmentation cavity to push downwards over the sides of the egg. As a consequence only a short portion of the head lies in front of the dorsal lip which moves backwards very little (if it advances at all), and the material of the sides lies along the blastoporic rim that encircles the equator of the egg, or may even lie above the equator. It is this possibility that HERTWIG has, I believe, not fully appreciated, and which has lead him, as will be pointed out below, to confuse this result with the formation of a whole embryo of a different type.

Several of the preceeding modes of abnormal development may appear to a greater or less extent in the same egg, and thus render the results correspondingly complicated²). Furthermore we should bear

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¹) This series No. V.

²) Extreme forms of spina bifida (ring-embryos) are also due to the lack of downgrowth of the material of the top of the egg. Ordinary spina bifida embryos are due to lack of overgrowth of one or of both sides of the normally placed lips of the blastopore. All combinations of these two conditions occur.

in mind another possibility, namely, that if the contents of the uninjured blastomere become rearranged after the operation, a whole development more or less complete takes place. In the light of these statements I propose now to examine in some detail Hertwig's and Roux's view respecting the outcome of the operation of injuring one of the first two blastomeres.

A Criticism of Hertwig's Results.

Hertwig admits that in most of the eggs on which he operated cells were split off from the top of the injured blastomere, and were added to the cells of the developing half. My own observations also show that this occurs where Roux's operation is incompletely performed.

The segmentation cavity lies, according to Hertwig, sometimes at the edge of the developing half and is then bordered, in part, by the injured material, and sometimes it lies well within the cellulated part. The former condition would arise when the segmentation has been confined to the uninjured blastomere; the latter when segmentation has taken place also in the upper part of the injured blastomere. It is also to be recalled that if a rotation of the contents of the uninjured blastomere were to take place the segmentation cavity would come to lie some distance from the injured half, as Wetzel has shown.

If the contents of the injured blastomere has rotated the position of the dorsal lip of the blastopore will also be affected, according to the extent and direction of the rotation. The results of Schultze and of Wetzel show how varied the position of the dorsal lip may be in different eggs, and even in the two parts of the same egg. Under these conditions the blastopore might lie far removed from the injured half, and then be able even to complete its formation, and, perhaps, even its closure, without coming into contact with the line of separation between the injured and the uninjured parts; but this will seldom occur, since the blastopore is likely to meet with the injured part on one or the other side.

If the protoplasm has not rotated, the dorsal lip will appear, as already explained by means of diagrams, *B* and *C*, near the line of demarcation between the injured and uninjured parts. The embryo that develops will come to lie more or less obliquely on the egg.

Still another possibility must be taken into consideration. The failure of the material of the upper hemisphere to be carried downwards during the cleavage may cause the head-end of the embryo to appear high up on the egg as shown in Figs. 4 and 10. The head will then lie more or less asymmetrically on the dark part, and its posterior end will be continuous with a ring around the dark field that may develop as a blastoporic rim and represent the sides of the embryo. Embryos of this sort may appear as whole embryos of half-size resulting from a rotation of the protoplasm, but theoretically there are important differences between the two kinds of embryos, for, while in the former the blastoporic rim would not usually encircle the whole yolk (although such a condition might occur in one form of development owing to a blocking of the blastopore by the injured half), in the other case the whole black-white rim represents the blastopore (which may be imperfect on one side if the effects of the injury extend into this region).

Hertwig's figures show that many of the embryos that he has found belong to the one or to the other of these categories. It would, I think, be possible to refer each one to its type, although without more complete information in regard to each case, and to the immediate results of the operation; and further without a knowledge of the complete series of sections, it would be somewhat hazardous to make this attempt.

Let us next examine Hertwig's interpretation of his results. He claims that none of his operations gave a half-embryo in Roux's sense, yet one case appears to be very nearly a half embryo (as Roux has pointed out), and others approach this condition, as shown by the sections that Hertwig gives. Since in nearly all the cases that Hertwig describes contributions have been made to the developing half from the injured blastomere it is not surprising to find that most of his embryos are somewhat more than half, especially at the anterior end.

In regard to the gastrula stages Hertwig's position is summarized in the following italisized statement on pages 752—753: »Ich sehe in ihnen nichts anderes als Gastrulae, die in abweichender und mehr oder minder gestörter Weise entwickelt sind. Die Ursache aber, welche die gestörte Entwickelung hervorgerufen hat, ist der Dotter, der neben der zur Gastrula sich umwandelnden zelligen Eihälfte im Eiraum noch vorhanden und theils durch den äußeren Eingriff zur Gerinnung gebracht und abgetödtet, theils aber auch noch leidlich in seiner ursprünglichen Beschaffenheit erhalten ist, wie er denn hier und da nachträglich in Zellen zerlegt worden ist. Dieses geschädigte Dottermaterial ist an die sich entwickelnde Eihälfte dicht gepresst, ja geht stellenweise continuirlich in sie über, und übt auf den Entwickelungsgang einen ähnlich modificirenden Einfluss aus, wie bei den meroblastischen Eiern der Nahrungsdotter auf den sich zum Embryo umbildenden Theil. Die zerstörte Substanz nimmt später die ventrale und hintere Gegend der Gastrula ein und ist die Ursache, dass in diesem ganzen Bereiche ein äußeres Keimblatt fehlt. Wenn die nicht in Zellen zerlegte Dottermasse aus der Eihülle hätte ganz entfernt werden können, so würde die aus der überlebenden Hälfte entstandene Gastrula den Defect im äußeren Keimblatt nicht aufweisen, mit anderen Worten, wir würden eine Gastrula wie in den Experimenten von Driesch erhalten haben, die sich lediglich durch eine geringere Größe von einer normalen Froschgastrula unterscheiden würde.«

This statement embodies a conception of the results of the operation that is in principle fundamentally different from my own point of view. The claim that the »injured yolk material . . . exerts on the developmental process an influence similar to that which the food-yolk of the meroblastic egg has on the material that goes to form the embryo« is not borne out by the description of the embryos that are described, and gives, I believe, a totally wrong impression of the relation of the injured to the developing part. Instead of being a constituent part of the developing embryo as is the yolk of a meroblastic egg the injured yolk forms an obstacle to the normal path of development, preventing the extension of the blastoporic lips, and in consequence causing abnormalities in the development. The kinds of changes induced have been already sufficiently described, and from this account it will be clear that the two points of view have nothing in common.

The further statement in this quotation, namely, that »the injured substance takes later a posterior and ventral position« is by no means accurate. The position held by the injured part is a necessary consequence of its position and will vary in the different types of embryos. The injured part holds throughout its original relation to the uninjured part, except in those cases where a rotation of the protoplasm has taken place, when a whole embryo of half-size is produced. Even in this latter case the injured part can not be described as having any such relation to the developing part as has the embryo of a meroblastic egg to the yolk, but it acts simply as an impediment to the normal extension and overgrowth of the blastopore. Consequently any resemblance between the two processes is entirely superficial.

The final statement in the quotation may or may not be correct, for, it has not been possible up to the present time to remove in the frogs egg one of the first two blastomeres. In other cases in which the operation is possible it has been found that in some instances an incomplete embryo develops (as in the ctenophore), in others a whole embryo of half size (as in the teleost). It is not impossible that even in the frog's egg, if the contents of one of the first two blastomeres could be removed and the remaining blastomere kept in its normal position there might still be found in evidences of the half structure in the appearance of the blastoporic rim, for, if not, we should have to suppose that the superficial material that goes to form the dorsal and ventral lips extends around the »raw« side of the blastomere after its isolation and restores the necessary material to

Diagram *D*.

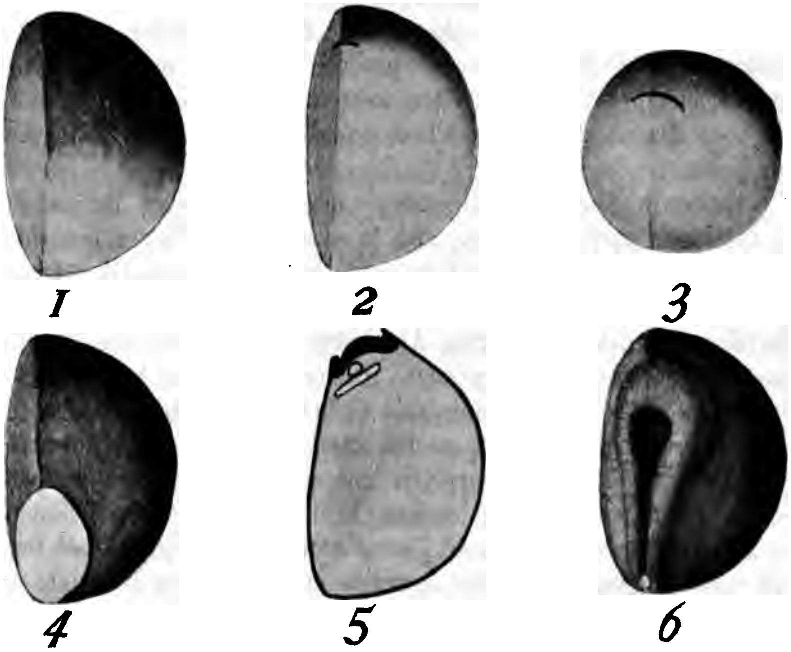

Fig. 1. One blastomere, other supposed to have been removed. Fig. 2. Gastrulation of same, dorsal lip represented as extending over on to the contact surface. Fig. 3. Representing the contact surface as bulging out to complete sphere. Fig. 4. Later stage of Fig. 2. Fig. 5. Cross-section of Fig. 6. Fig. 6. Representing embryo forming partly on old surface and partly over contact surface. All figures entirely imaginary.

form the embryo on that side of the egg at which the blastomere was in contact with its fellow. Such a possible change is illustrated in Diagram *D* 1, 2, 3. Whether this would take place completely or even partially (Diagram *D* 4, 5, 6) we have no means of judjing at the present time, and probably on some such change as this would depend whether a whole or a half development would occur. HERTWIG's general point of view would lead one to suppose that he imagines the contact surface in itself capable of producing the missing half without a readjustment of the materials, but this seems to me highly improbable. In the case of the salamander the isolated blastomere may in some cases produce a whole embryo, but the internal changes that take place in it after its removal have not as yet been described, and the whole question here raised turns on just this point.

HERTWIG found that in many cases the embryos lie obliquely on the black part of the egg, and in most of these cases one side of the medullary plate is less developed than the other. He refers this result to the asymmetrical position of the gastrula invagination: »In dem in Zellen zerlegten Abschnitt des Eies ist die Gastrulaeinstülpung asymmetrisch erfolgt. Daher kommt bei Ausbildung der Urmundnaht die Verwachsungslinie nicht in der Mitte zu liegen, sondern ist auf der einen Seite an den Rand des Zerstörungsfeldes näher herangerückt. Die Medullarplatte entsteht aber aus dem zu beiden Seiten der Urmundnaht gelegenen Bezirk des äußeren Keimblatts. Da nun der an den zerstörten Dotter unmittelbar angrenzende Bezirk sich unter ungünstigeren Bedingungen als der andere befindet, wird die auf ihm entstehende Rückenmarkshälfte in der Entwickelung etwas zurückbleiben und eventuell kümmerlich ausfallen müssen. Mithin können wir sagen, dass die Ungleichheit in der Ausbildung der Nervenplatte in der Art der Gastrulation schon begründet ist. Mit einer Postgeneration haben diese Fälle nichts zu thun.« The explanation would be more satisfactory of the asymmetrical position of the gastrula invagination, on which the asymmetry of the embryo is supposed to depend, could be accounted for. In reality this asymmetry of the embryo is due in part to the incomplete segmentation of one side of the egg in most cases, but in those eggs in which the contents of the blastomere has rotated after the operation the asymmetry is due primarily to this, and to the consequent interference with the advance of the dorsal lip over the surface. In these two ways the asymmetry of the embryo can be accounted for, and, I think, such an explanation will recommend itself as preferable to Hertwig's vague suggestion that the result is due to the asymmetry of the gastrulation process.

How different Hertwig's point of view is from my own may be gathered further from the following statement (page 770): »Nach meinen Versuchen entsteht auch nach Zerstörung eines ansehnlichen Theils des Froscheies aus dem entwickelungsfähigen Rest immer noch ein leidlich normaler Embryo und zwar auf directem Wege, das heißt durch Vorgänge, die sich im Rahmen des normalen Entwickelungsprocesses bewegen. Dabei wird die ungetheilte Substanz an Orte geschafft, wo sie am wenigsten störend in die Organisation des Ganzen eingreift, woraus sich auf eine gewisse Zweckmäßigkeit im Entwickelungsverlaufe, auf eine Anpassung desselben an die veränderten Verhältnisse schließen lässt. Das ist natürlich nur möglich, wenn der entwickelungsfähige Eirest eine Substanz ist, welche in ihren Eigenschaften dem Ganzen gleicht, und nicht eine Substanz, welche nur einen mit besonderen Eigenschaften begabten Theil des Ganzen darstellt, wie es die Auffassung von Roux ist. Nach dieser entgegengesetzten Auffassung kann sich ja an einer der beiden oder einer der vier ersten Furchungszellen des Froscheies immer nur eine Hälfte oder ein bestimmtes Viertel des Embryo entwickeln, so dass Zerstörung von zwei Vierteln immer einen entsprechenden Verlust von Organen des Körpers bedingt, entweder einer ganzen Gegenseite, oder der vorderen oder hinteren Rumpfhälfte.«

How the injured yolk could be placed by the egg in that region where it can produce the least injurious effect on the course of development is by no means clear. Certainly such a process would be »zweckmäßig« to a marvellous degree. In reality the »adjustment« is a simpler and less teleological process that Hertwig supposes.

The Question of Anterior and Posterior Gastrulae and Embryos.

Roux claimed that when the two blastomeres of the first four that lie on the darker side of the egg are injured or killed the other two produce the anterior end of an embryo. Hertwig has discussed the possibility of the formation of such embryos, and has given a different interpretation to Roux's results. Since this discussion brings out very clearly certain changes that Hertwig supposes to take place after the operation, and since I have found that

Diagram *E*.

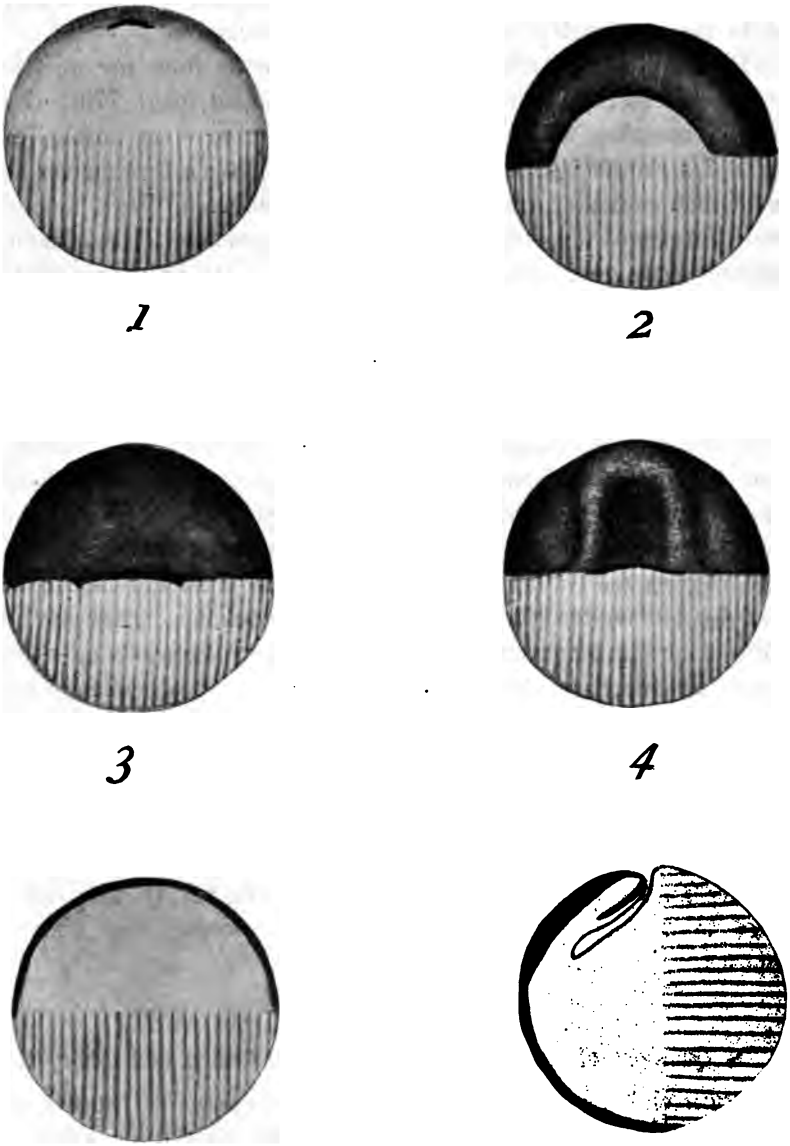

Fig. 1. Gastrula stage of egg in which two posterior blastomeres have been injured. Fig. 2. Later gastrula of same. Fig. 3. Represents dorsal lip as having advanced to injured half. Fig. 4. Anterior half-embryo. Fig. 5. Section through Fig. 4 in plane of paper. Fig. 6. Section through Fig. 4 in median plane of embryo.

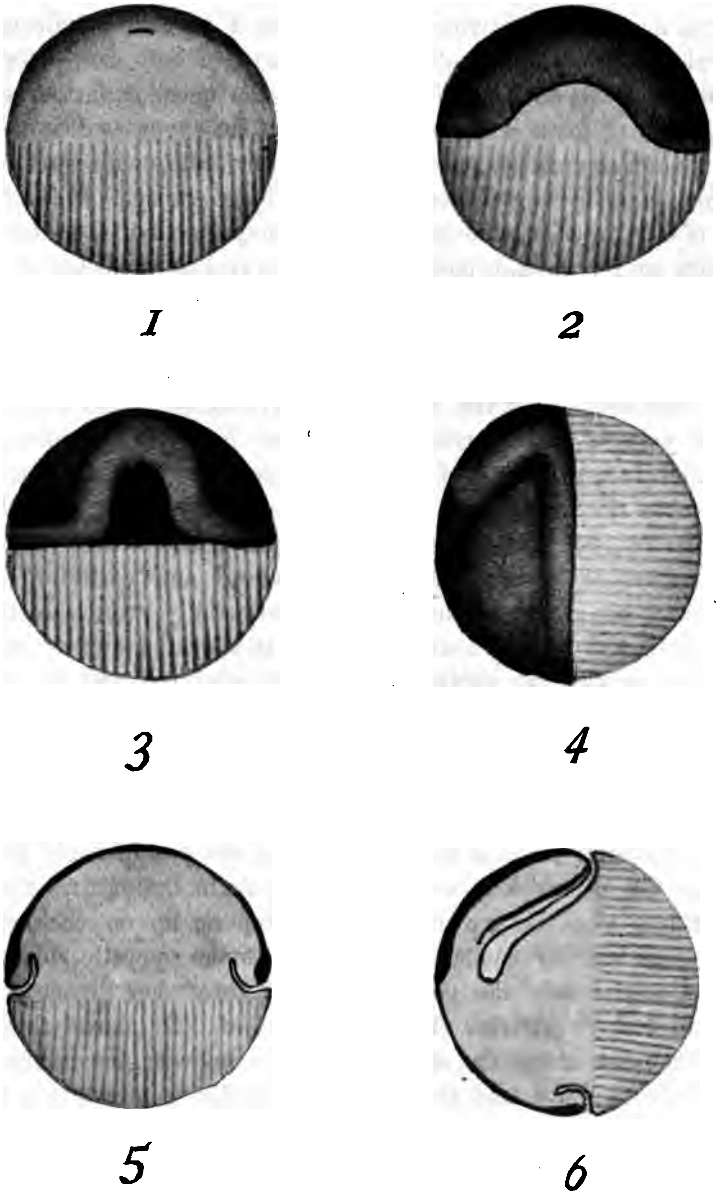
Diagram *F*.

Fig. 1. Gastrula stage of egg in which two posterior blastomeres have been injured. **Fig. 2.** Imaginary stage where the lateral lips are represented as extending around the edge between the injured and uninjured halves. **Fig. 3.** Imaginary embryo derived from last stage. **Fig. 4.** Side view of last. **Fig. 5.** Imaginary section of Fig. 3 in plane of paper. **Fig. 6.** Imaginary longitudinal section of Fig. 3. Compare these figures with those Diagram *E*.

anterior embryos may be produced, and, further, since it is possible that the one figure given by ROUX to illustrate his embryones anteriores may, as HERTWIG claims, and as I have also intimated on several occasions, represent a whole embryo of half size — for these reasons it seems to be desirable to give the question further sifting.

HERTWIG gives a description of what he supposes ROUX means by an anterior embryo, and this description tallies with what, in my opinion, is a correct interpretation of such an embryo. In the formation of an anterior embryo we must suppose that the dorsal lip appears in its normal position; the lateral lips then begin to extend, as normally, around the sides of the lower part of the egg, but they soon abut against the wall of injured material as shown in Diagram *E* 1, 2. If now the dorsal and lateral lips begin to grow backwards over the yolk their advance will also be stopped by the yolk of the injured half, Diagram *E* 3. It is important to note that in an embryo of this sort there is no continuation of the blastoporic rim around the egg between the developing and the injured halves, nor can any cause be assigned why such a rim should be expected to develop. The postero-lateral and posterior lips of the blastopore are to be thought of as potentially present in the injured half, and therefore undeveloped. If they did appear in these embryos, around the border between the uninjured and the injured parts, the embryo would correspond to a whole embryo of half size with the condition of spina bifida behind, Diagram *F* 3, 4; and it appears that this is HERTWIG's conception of the only possible form of such embryos. From my own point of view such a condition could only arise when a rearrangement of the protoplasmic parts of the egg has occurred at an early stage, as in SCHULTZE's reversed egg, where also the growth of the blastoporic lip on each side is interferred with by coming in contact with the opposite side of the egg. Unfortunately the only figure that ROUX has given to illustrate an embryo anterior (ROUX's Fig. 6 Taf. VII) shows thickened ridges running along the sides of the egg between the injured and the uninjured parts, and these ridges look like continuations of the medullary folds. The embryo is similar to the imaginary one shown in my diagram *F* 3, 4.

HERTWIG's difficulty in understanding how an anterior embryo could form I do not understand, and his account of what would take place when the two posterior blastomeres are injured (as given on pages 750—751) appears to me confused in the extreme.

HERTWIG finds even a greater difficulty in understanding how a posterior gastrula could possibly arise when the two anterior blastomeres are injured. This difficulty lies, it seems to me, in a misconception of the meaning of a dependence of the different parts of the egg on each other. More recent results, especially those on the *Ctenophore* and on *Cerebratulus* and *Dentalium*, show that isolated blastomeres may differentiate into the same organs that they would have formed had they remained in contact with the others. This same process may occur to a more limited extent in the frog's egg, in which, after injury to certain parts, others may continue to differentiate along the original lines. HERTWIG's position is summed up in the following italized statement: »Es bleiben daher nur noch die hinteren Halbgastrulae übrig, deren Entstehung nicht minder an ganz außerordentliche Bedingungen geknüpft sein müsste. Denn da die vordere Gastrulahälfte zeitlich am frühesten durch Einstülpung entsteht und die hintere sich nur im Anschluss daran durch Fortsetzung der einmal gebildeten Einstülpung nach hinten entwickelt, so würde es sich bei dem Zustandekommen hinterer Halbgastrulae um die sonderbare Erscheinung handeln, dass ein in seinem Beginn unmöglich gemachter Entwickelungsprozess doch in seinem weiteren Fortgang ermöglicht ist, oder dass der Anfang der Einstülpung fehlt, ihr Weiterwachsthum nach hinten aber stattfindet.« »Niemals habe ich solche Bildung gesehen, jedenfalls gehören die von mir oben beschriebenen Formen auch nicht in diese Categorie. Auch ROUX gibt an, ‚von den entsprechenden hinteren Halbbildungen habe ich keine sicheren Exemplare. Eine der vier aufgehobenen Semigastrulae ist vielleicht wegen der Dicke und Kürze der Urmundslippe als eine Posterior anzusprechen‘.«

My own experience, based on a large number of experiments to examine this point, shows, it is true, that the posterior part of the blastopore much less frequently appears after injury to the two anterior blastomeres than does the anterior part of the blastopore after the destruction of the two posterior blastomeres. Furthermore, since even in these cases, in which the blastopore appears, further development does not proceeds to any extent it is difficult to identify with certainty the kind of structure that is formed. Nevertheless, I think, that the following considerations leave but little doubt that gastrulae posteriores may arise. First, in several cases in which the two anterior blastomeres had not been seriously injured so that they showed only a retarded development the blastoporic rim of the posterior parts continued into that of the anterior part where indications of the formation of the anterior end of the blastopore sometimes appeared. Second, if the blastoporic rim that appeared in other cases on the posterior half was in reality an anterior end, then we should expect it to have shown the characteristics of that end, since these always appear in the case of anterior embryos. Third, from analogy with SPEMANN's results on the salamander we should conclude that posterior gastrulae may arise. He found that when the egg of *Triton* was constricted by a ligature at the two-cell stage (in those cases in which the first cleavage plane corresponds to the cross-plane of the body) indications of the posterior lips of the blastopore appeared on the posterior half. The posterior portion does not, however, like the anterior portion continue to differentiate.

There is also a further consideration. The first plane of division of the frogs egg not infrequently fails to coincide exactly with the middle plane of the embryo, and in extreme cases may be even 90 degrees away from it. Under these circumstances the second plane of cleavage must also bear a variable relation to the median plane of the embryo. Not infrequently, therefore, the two posterior blastomeres will probably contain more of the posterior part of the embryo on one side and less on the other. Whether the blastoporic rim is more likely to appear under such circumstances remains to be shown¹). The main question is not however altered by this consideration.

I conclude, therefore, that the posterior and lateral-posterior parts of the blastoporic rim may appear when the anterior half of the egg does not develop. The failure of the posterior lip to differentiate further can only be accounted for as due to the absence of the anterior parts. Unless the anterior parts of the medullary folds are present the stimulus to form the posterior folds is absent. In this connection it is important to note that the process of concrescence of the lateral lips takes place from before backwards.

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¹) The preceeding account was written during the summer in California. On my return I found an interesting paper by BRACHET (Arch. de Biologie. XXI. 1904) in which the author shows that when the posterior cells of an egg of the kind just described is operated upon more than half an embryo develops from the uninjured blastomere. Thus the conclusion at which I had arrived on theoretical grounds is established by actual experiment.

Hertwig's spina bifida embryos. At times Hertwig found that spina bifida embryos arose after he had punctured one of the first two blastomeres. He explains their origin as due to the principal injury being to the lower hemisphere, although he had punctured the blastomere in its upper part. His figures, especially his sections, show that these embryos are often defective on one side to a greater extent than on the other, and approach, therefore, the condition of hemi-embryos. I have found that this is not an uncommon result when one of the blastomeres is only partially injured. The failure of the injured side to develop is due, no doubt, in part to the failure of the blastoporic rim to properly form, and, even if it forms at times, to the failure of the yolk on that side to pull in and allow the lip to advance. These embryos, instead therefore of being entirely different from the others form a continuous series with the other kinds 1), according to my interpretation; while, according to Hertwig, there is a fundamental difference in type or mode of development in these cases of spina bifida and in those in which the uninjured blastomere alone develops. Hertwig's interpretation of how these embryos arise is essentially the same as that which I should give, namely, to injuries of the yolk region causing a failure in part in the closure of the lateral lip or lips of the blastopore. One of the ways in which the yolk may be affected by the needle is as follows. The path of the needle may be oblique and the nucleus of the injured side come to lie above the injured region which acts as a partial barrier to the normal migration of nuclei from the upper region of the cell to the lower parts. In consequence of the deficiency in nuclei the yolk may fail to withdraw normally, and thus remain protruding in the blastopore-mouth. If the puncture is deep the needle may also injure the yolk part of the other blastomere. Hence the whole lower part of the egg might fail to draw inwards when the lateral lips of the blastopore are formed. The result would be the condition of spina bifida.

Roux's postgeneration of half-embryos. There is a theoretical difficulty that Roux's description of postgeneration of half embryos meets with that has not, I think, been sufficiently appreciated, but which in the light of more recent results, in respect to the value of germ-forming areas, is so serious that, aside from other arguments 2),

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1) Except in those cases where an early rearrangement of the protoplasm has occurred.

2) See the papers of C. Ziegler, and Morgan and Torelle.

there is raised a question of grave doubt as to the possibility of such a process occurring. According to Roux's view all the posterior region of the frog's embryo behind the brain is formed by the inralling of the lateral lips of the blastopore. Therefore if only half an embryo is formed its side will abut against the yolk hemisphere of the injured side, i. e., against that material that in the normal embryo goes to form the floor of the archenteron. This is shown in surface view in Diagram *B* 3 and in section in Diagram *B* 4. It is this material which, in some mysterious way, »under the influence of the developing half« is transformed into the missing half of the medullary plate, of the mesoderm and of the wall of the archenteron. That such a process could ever occur, and that in some cases it took place within half of a day is too improbable to believe.

Diagram *G*.

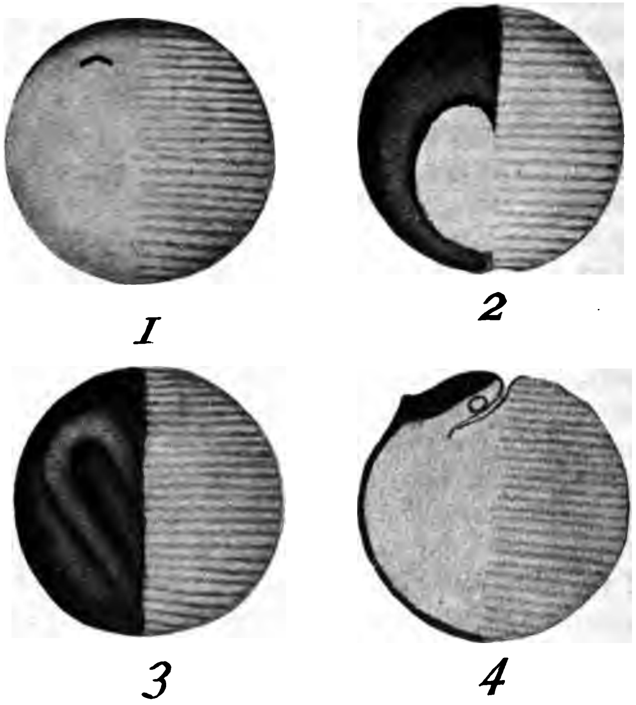

Fig. 1. Gastrula stage of egg in which one of the first two blastomeres has been injured and in which the dorsal lip lies not at the edge but within the uninjured blastomere. Fig. 2. Later stage of same. Fig. 3. Embryo derived from gastrula of this type. Fig. 4. Cross-section of same. More than half an embryo is formed in the anterior region of the embryo.

There is another possibility that might be considered in this connection. The anterior end of the embryo is formed out of the material anterior to the first position of the dorsal lip, and even in the half-embryos the anterior end is laid down more nearly as a whole than as a half structure. It may be imagined that during the process of »postgeneration« the missing part grows backwards from this anterior material over the yolk of the injured side. This superficial backgrowth could be readily observed if it occurred, but so long as no such observations have been recorded it is safe to assume that it does not occur[1]).

There is still another possibility to be reckoned with. If the dorsal lip of the blastopore should not appear exactly along the edge between the uninjured and the injured half (either because it lies somewhat further to one side, or because some of the material of the injured half has become cellulated) but more in the uninjured part as shown in Diagram *G* 1, 2, 3, 4, then as it extends backwards a little of the material that belongs to the missing half of the embryo may also extend backwards, and thus contribute to the formation of the half on the injured side, Diagram *G* 3, 4. In fact I have often observed a little mesoderm on the »outer« side of the notochord. From this material we might imagine a rapid regeneration of the missing half might take place. Theoretically there would be less difficulty in explaining the sudden reappearance of the missing half in this way than in any other way so far suggested. It is true we should still have to account for a new connection being formed with the yolk of the injured side, (see Diagram *G* 4), and for the growth of the half sized notochord into a whole one, and for the closure of the archenteric slit along the exposed edge, etc., but even if all this is theoretically possible we lack completely any sufficient observations to show that it occurs; and there is some evidence to show that in most cases, at least, these embryos do not complete themselves, but remain in approximately the same condition as when first formed. I am forced to conclude, therefore, that even in this way the process of »postgeneration« does not take place. The only cases, in fact, in which the »missing« half is formed from the injured half are those in which this half has been so little injured that it is capable of gastrulating when the other half gastrulates — or at most slightly lags behind that half.

HERTWIG also denies the possibility of ROUX's postgeneration, but as he bases his criticism on what he himself supposes to take place in the egg after injury, and as I think this interpretation is largely erroneous[1]), I can not accept his criticism, although I agree with HERTWIG that the postgeneration as described by ROUX does not in all probability take place.

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[1]) The backgrowth of ectoderm is said to take place.

ROUX's Hemiooplastic Embryos. In later publications ROUX described another method of development of embryos from the uninjured blastomeres. These embryos he calls hemiooplasten. Their most peculiar feature is that they are supposed to develop without making use of the material of the injured side, or at least only to a very small extent.

This type of embryo, ROUX believes, does not differ essentially from those in which the missing half is postgenerated, because he finds all intermediate conditions between those that make use of all of the material of the operated blastomere and of those that make use of little, or even of none at all of this material. He supposes, in fact, that even in these hemiooplastic embryos a half-embryo first develops, which then completes itself laterally by a sort of regenerative process, that involves, in the main, or entirely, its own substance. It has puzzled me a great deal to discover what kind of embryos ROUX has found to which he gives such an impossible name. I can not but agree with HERTWIG's criticism that it is improbable under the conditions that a half embryo could first be formed that then regenerates its missing half out of its own material. On the other hand I do not question that ROUX has found whole embryos of half-size; it is his interpretation of how they develop that seems to me improbable. It appears more probable that these embryos must have arisen in one of the following ways. A rotation of the contents of the uninjured blastomere would cause that blastomere to produce a whole embryo by morphallaxis, i. e., without any regeneration in the ordinary sense (»restorative regeneration«) taking place. If at the same time a constriction of the developing half should separate it from the injured half the result would be further simplified. Something of this sort I saw, but did not sufficiently study by means of sections, in the egg of *Rana esculenta* that I used at Naples, in which by rotating the egg, after operating on one blastomere, the uninjured blastomere gave a whole embryo. I have also occasionally found in the embryos of *Rana palustris* that when the yolk of the injured cell is so nearly completely lost, either at the time of the operation or later, that the uninjured half developed almost alone, the embryo approached, although never completely, the condition of a whole embryo. As explained above such a condition may have arisen not through a rotation of the contents, since this occurs with difficulty in this species, but from the egg being one of these exceptional cases in which the crescent lay not at the edge, but nearer the middle, of the uninjured blastomere. In most cases however when the contents of one blastomere is largely lost a half embryo developed, as I have described in No. IV of this series. In neither of the two ways suggested above in which a whole embryo may arise is there any need to suppose a half first develops, which then proceeds to »postgenerate« the missing part.

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[1]) Less so, however, in those cases in which a rotation of the contents of the uninjured blastomere take place.

Summary.

1) Experiments undertaken to study the effects of incomplete injury to one of the first two blastomeres of the frog's egg show that the operation may involve only the outer part of the injured blastomere (Diagram *A* 1), or the outer and part of the lower portion (Diagram *A* 2), or the outer and all of the lower portion (Diagram *A* 3). The embryos that develop in the first case may be normal except for deficiencies on one side; in the second case more than half an embryo may be formed (Diagram *C* 3); and in the third case a half embryo (Diagram *B* 3).

2) Some of the different forms of embryos actually obtained are represented in Plate XVII Figs. 1—12. Most of these can be referred to one or to another of the above types, or combinations of them. (See also 7.)

3) An analysis of the conditions that would result if one of the first two blastomeres could be completely removed (Diagram *D* 1), leads to the conclusion that unless the more superficial material could extend over the old contact-surface no portion of an embryo could develop out of the material exposed at this contact surface, and only a half-embryo could develop on the original surface of the blastomere; but if the surface material did extend over the contact surface a whole embryo might develop that would be more or less perfect on the »new« side in proportion to the extent to which the movement of surface material has taken place (Diagram *D* 2—6).

4) When the two posterior blastomeres are injured the dorsal lip of the blastopore extend over its half of the lower (half) hemisphere as shown in Diagram *E* 1—3. This leads to the type of embryo represented in Diagram *E* 4—6. The lips of the blastopore should not be expected, in this case, to extend around the line between the uninjured and injured halves.

5) The series of figures represented in Diagram *F* 1—6 shows, in contrast to the last, what would take place if the lateral lips of the blastopore did extend around the line between the uninjured and injured halves. No reason can be given why we should expect that the lateral lips would extend in this way when the two posterior blastomeres are injured, unless further changes take place that are not the immediate outcome of the operation. It is probable that the embryos that have been described as embryones anteriores, and which belong to this type, are not anterior embryos at all, but whole embryos with the condition of spina bifida behind. This type of embryo may arise in one of two ways, either as the result of a rearrangement of the contents of the uninjured blastomere after the operation, so that a whole embryo is formed (the closure of the blastopore of which is interferred with by the injured blastomere); or somewhat similar embryos to this may arise when the embryo-forming-material at the top of the egg fails to become carried to the equatorial regions during the cleavage. If only the lower portions of the blastomere operated upon have been injured this type of embryo may have both its half medullary plates present extending around the sides, but if more of the upper portions also of the blastomere have been injured the half medullary plate may be perfectly developed only on one side.

6) Observations have shown that the dorsal lip of the blastopore often fails to correspond to the first plane of cleavage and may lie more in one or the other blastomere. If in an egg of this sort the location of the dorsal lip lies within the uninjured blastomere (Diagram *G* 1) then more than a half-embryo may develop (Diagram *G* 2—4).

7) A comparison of my results with those of Hertwig leads me to a point of view very different from that maintained by him. The different forms of embryos described by Hertwig have also been met with in my experiments and can be accounted for, either (1) as a result of incomplete injury to the blastomere operated upon; or (2) as due to a rotation of the contents of the uninjured blasto-

mere after the operation; or (3) as due to the failure of the yolk-portion to become drawn into the blastopore, or (4) as due to failure of the material of the top of the egg to become carried to the equatorial regions during the cleavage stages. I find no evidence in favour of HERTWIG's view that the injured yolk behaves like the yolk of a meroblastic egg. The yolk forms simply an obstacle to the advance of one or another part of the blastopore according to the location of the injured region. There is no evidence that the yolk is carried into a posterior ventral region as a sort of »adaptive« process to the new conditions.

8) There are serious difficulties in the way of ROUX's hypothesis that the missing half of the embryo can be formed out of the injured blastomere — »postgenerated« — after a half-embryo has been formed on the uninjured half. The evidence that such a process can take place at such a time is insufficient, and on theoretical grounds it does not seem possible that such a process can occur. The delayed development of the injured blastomere (if the delay is not prolonged beyond the gastrula-stage) may account for the belated development of the missing half of the gastrula, but after the half-embryo has been formed it is improbable that the injured half ever supplies the missing half, either by the normal process of gastrulation or by a new method of development; for, if by the normal method the belated blastopore should be seen to appear and to advance over the yolk, and no such process has yet been described; and if not in this way the embryo-forming-material of the injured half is too far removed from the half-embryo to be used and no one will wish to maintain that out of the yolk, lying opposite the half embryo, the dorsal portions of the embryo could be developed.

Zusammenfassung.

1) Versuche zum Zwecke des Studiums der Wirkungen, welche unvollständige Verletzung der einen der beiden ersten Blastomeren hervorbringt, zeigen, daß die Operation vernichten kann: entweder nur den äußeren Teil der verletzten Blastomere (Diagramm *A* 1), oder den äußeren und einen Teil des unteren Eiabschnittes (Diagramm *A* 2), oder endlich den äußeren und den ganzen unteren Abschnitt (Diagramm *A* 3). Die im ersten Fall entwickelten Embryonen können bis auf Defekte an einer Seite normal sein; im zweiten Fall kann mehr als ein halber Embryo entstehen (Diagramm *C* 3) und im dritten Fall ein Halbembryo (Diagramm *B* 3).

2) Einige von den wirklich erhaltenen verschiedenen Embryoformen sind auf Taf. XVII Fig. 1—12 abgebildet. Die meisten von diesen können auf eine

der oben erwähnten Typen oder auf Kombinationen derselben bezogen werden. (Siehe auch 7.)

3) Eine Analyse der Verhältnisse, die sich nach völliger Ausschaltung der einen der beiden ersten Blastomeren ergeben (Diagramm *D* 1), führt zu dem Ergebnis, daß ohne Ausdehnungsmöglichkeit des oberflächlicheren Materials über die alte Berührungsoberfläche kein Teil eines Embryos aus dem auf dieser Berührungsfläche exponierten Material entstehen kann, und lediglich ein Hemiembryo an der ursprünglichen Oberfläche der Blastomere sich zu entwickeln vermag; aber bei Ausdehnung des Oberflächenmaterials über die Berührungsfläche kann sich ein Ganzembryo entwickeln von größerer oder geringerer Vollständigkeit auf der »neuen« Seite nach Maßgabe der Ausdehnung, in welcher die Verschiebung des Oberflächenmaterials stattgefunden hat (Diagramm *D* 2—6).

4) Werden die zwei hinteren Blastomeren verletzt, so dehnt sich der dorsale Blastoporusteil über seine Hälfte der unteren (halben) Hemisphäre aus (vgl. Diagramm *E* 1—3). Das führt zu dem Embryotypus von Diagramm *E* 4—6. Man sollte in diesem Falle von den Blastoporuslippen nicht erwarten, daß sie sich rund um die Grenze zwischen den verletzten und den intakten Hälften ausdehnen.

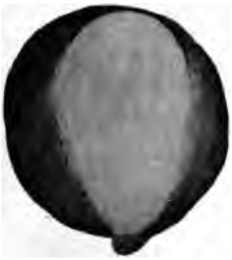
5) Die Figurenreihe 1—6 in Diagramm *F* zeigt im Gegensatz zur vorigen die Vorgänge bei Ausdehnung der lateralen Blastoporuslippen rund um die Grenze zwischen verletzter und unverletzter Hälfte. Eine solche Ausdehnung der lateralen Lippen können wir nicht erwarten bei einer Verletzung der beiden hinteren Blastomeren, ohne daß weitere Veränderungen Platz greifen, die von der Operation nicht unmittelbar bedingt sind. Wahrscheinlich sind die als Embryones anteriores beschriebenen, unter diese Kategorie fallenden Embryonen gar keine solchen, sondern Ganzembryonen mit Spina bifida im hinteren Teil. Dieser Embryonaltyp kann auf zweierlei Weise entstehen, entweder als das Ergebnis einer Wiederordnung des Inhalts der unverletzten Blastomere nach der Operation, woraus ein Ganzembryo resultiert (dessen Blastoporusschluß durch die verletzte Blastomere verhindert wird); es können aber auch einigermaßen diesen ähnliche Embryonen entstehen, wenn das embryobildende Material am Eischeitel während der Furchung nicht in die Äquatorialregion gelangt. Sind lediglich die unteren Teile der operierten Blastomere verletzt worden, so können bei diesem Embryotypus seine beiden halben Medullarplatten da sein und sich um seine Seiten herum erstrecken; ist aber etwas mehr von den oberen Teilen der Blastomere verletzt worden, so kann die vollständige Entwicklung einer Medullarplatte auch nur auf einer Seite erfolgen.

6) Die Beobachtung hat gezeigt, daß die dorsale Blastoporuslippe mit der ersten Furchungsebene oft nicht übereinstimmt und mehr der einen oder andern Blastomere angehören kann. Wenn bei einem derartigen Ei die Stelle der Dorsallippe im Bereich der unverletzten Blastomere liegt (Diagramm *G* 1), dann kann sich mehr als ein Halbembryo entwickeln (Diagramm *G* 2—4).

7) Ein Vergleich meiner Ergebnisse mit denen HERTWIGS führt mich zu einem von dem seinigen sehr verschiedenen Standpunkt. Die verschiedenen von HERTWIG beschriebenen Embryoformen sind mir bei meinen Versuchen auch begegnet, und sie können aufgefaßt werden: 1) als das Ergebnis unvollständiger Verletzung der operierten Blastomere; oder 2) als hervorgebracht durch eine Rotation des Inhalts der unverletzten Blastomere nach der Operation; oder 3) als Folge der mangelhaften Einziehung des Dotterteils in den Blastoporus; oder 4) als Folge mangelhaften Transports des Materials vom Eischeitel nach der

Äquatorialgegend während der Furchung. Ich finde keinen Beweis für Hertwigs Ansicht, daß sich der verletzte Dotter wie der Dotter eines meroblastischen Eies verhält. Der Dotter bildet lediglich ein Hindernis für das Vorrücken des einen oder andern Teils des Blastoporus je nach der Lage des geschädigten Bezirks. Es gibt keinen augenscheinlichen Beweis, daß der Dotter nach einem hinteren ventralen Bezirk geschafft wird, als eine Art von »Anpassungsprozeß« an die neuen Verhältnisse.

8) Es bestehen ernste Schwierigkeiten für die Anwendung der Rouxschen Hypothesen, daß die fehlende Embryohälfte, nach der Bildung eines Halbembryos aus der unverletzten Hälfte, von der verletzten Hälfte aus — durch »Postgeneration« —, gebildet werden kann. Die Beobachtungen für den Eintritt eines solchen Prozesses zu dieser Zeit sind unzureichend und aus theoretischen Gründen erscheint dasselbe nicht möglich. Der Entwicklungsaufschub der verletzten Blastomere (falls sich der Aufschub nicht über das Gastrulastadium hinaus verlängert), kann als Ursache der verspäteten Entwicklung der fehlenden Gastrulahälfte gelten, aber nach der Ausgestaltung des Halbembryos ist es unwahrscheinlich, daß die verletzte Hälfte jemals die fehlende Embryohälfte liefert, sei es durch den normalen Gastrulationsprozeß, sei es durch einen neuen Entwicklungsmodus. Denn beim normalen Modus sollte der verspätete Blastoporus in seinem Auftreten und seinem Vorschreiten über den Dotter gesehen werden, und es ist kein solcher Vorgang bis jetzt beschrieben, und wenn der Vorgang ein andrer ist, so wird das embryobildende Material zu weit entfernt von dem Halbembryo, um direkt benutzt zu werden, und niemand wird wohl behaupten wollen, daß von den dem Hemiembryo entgegengesetzt liegenden Dottern aus sich die Dorsalpartien des Embryos entwickeln könnten.



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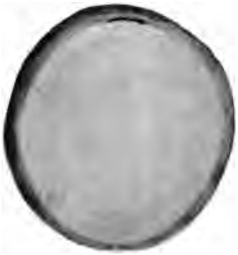

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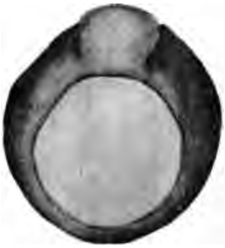

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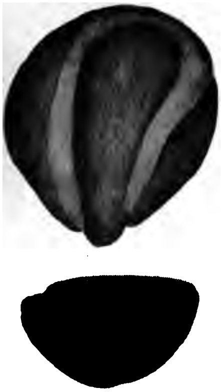

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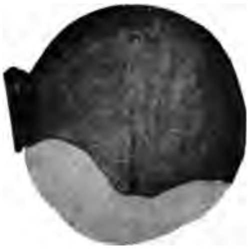

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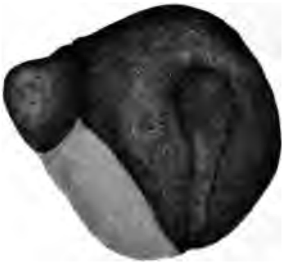

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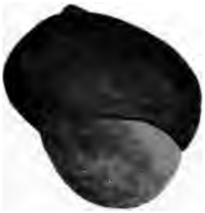

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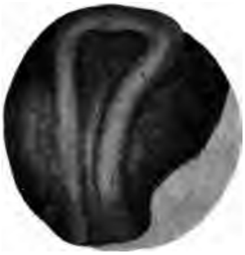

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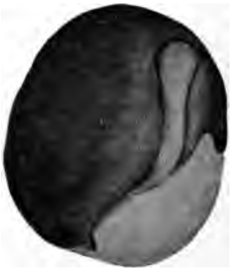

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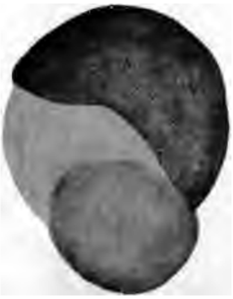

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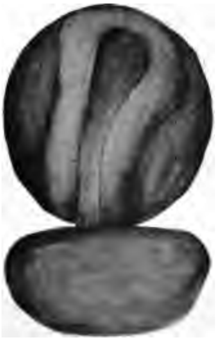

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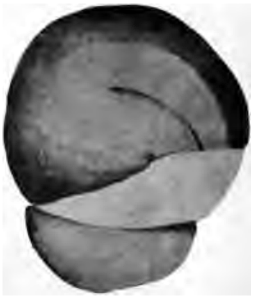

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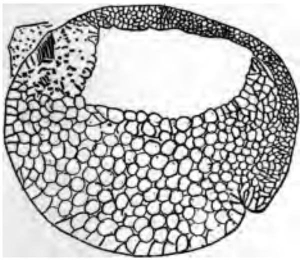
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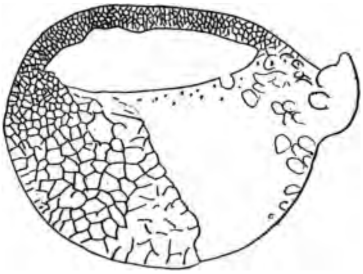
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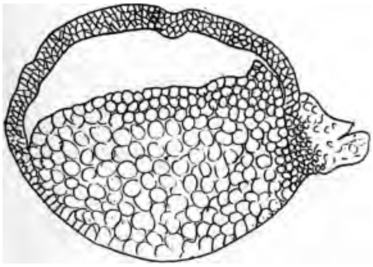
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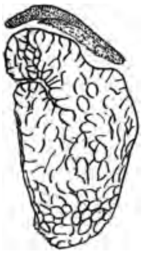
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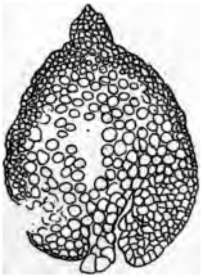
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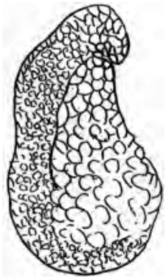
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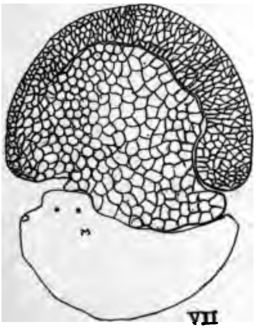
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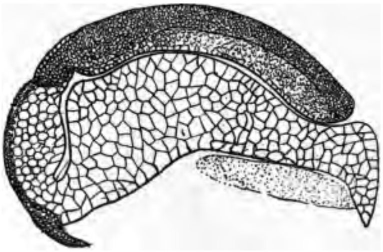
VIII

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The Relation Between Normal and Abnormal Development of the Embryo of the Frog:

VII. As Determined by Injury to the Top of the Egg in the Two- and Four-Cell Stages.

By

T. H. Morgan.

With Plates XIX and XX.

Eingegangen am 13. März 1905.

The results of the experiments described in the VI paper of this series, in which the upper four cells of the eight-cell stage were removed, suggested the idea that similar results might be obtained if the top of the egg at the two- or four-cell stages was injured or removed. The two experiments are not identical, for it is possible that movements of the material of the top of the egg might take place after the two- or four-cell stage and before the horizontal cleavage planes of the eight-cell stage are formed. There is however no evidence of such a movement to be seen from the exterior.

The presence of the horizontal cell walls at the eight-cell stage tends to limit the outflow of material to that contained in the upper eight cells when this region is punctured, while in the two- and four-cell stages regions some distance from the point actually stuck may flow towards, or out of, the opening. In fact, this is such a common occurence that the operation can not be recommended as one that is likely, in itself, to throw much light on the location of organ-forming-areas. For this reason the results described in this account serve best to illustrate other points in abnormal development, although, incidently, some light is thrown on the problem of regional differences, and a confirmation of the results of injury at the eight-cell stage has been obtained.

The outcome of the experiments recalls in many ways some of the early experiments of Roux in which the unsegmented egg of the frog was punctured in different regions. As I have pointed out elsewhere the results that Roux obtained even from the same kind of operation are often so different that no conclusions can safely be drawn from them in regard to the kinds of material contained in different parts of the egg. The discrepencies in the results are due to the difficulty of performing the same identical operation each time that the egg is injured, even when an attempt is made to puncture the same region. I have met with the same difficulties in my own experiments, but in these, owing to the presence of the furrows, the results of the operation can be more easily detected, and the great variety of results that immediatly follow the operation can be seen. Despite the fact that I attempted to puncture the egg at the top the outflow of material that followed the operation involved sometimes more of the posterior side of the egg, sometimes more of one or of the other lateral region of the egg, and sometimes, but less often, the anterior side. If the outflow is extensive, even the yolk from the center of the egg may push upwards and flow out of the opening, while the superficial material of the top, that is apparently more tenacious, loses relatively less of its substance. By heating the needle the injured region may be coagulated, and the outflow sometimes lessened, but in most of the cases here described a cold needle was used.

Results.

Many of the egg disintegrate completely if the injury is severe. If the injury is small and little or no substance protrudes, or is lost, normal development may take place. In most of the cases described in this paper the injury was quite extensive, and in the most extreme examples a half to three quarters of the contents of the egg may have been lost; yet in some cases the egg continued to develop as far as the gastrula stage. The following examples show some of the results of the operation.

Fig. 1 represents an embryo that developed from an egg stuck at the top with a cold needle in the two-cell stage. A wide gastrula opening is present. At one side the upper parts of the egg are more injured than on the other, and on the less injured side faint indications of a lateral extension of the dorsal lip are present. A longitudinal section through this embryo is shown in Fig. *A*. It is seen that the whole top of the egg has been destroyed, the yolk and the region of the dorsal lip alone being uninjured. In the region above the invagination ectoderm and mesoderm are present and continuous. All other parts of the embryo are absent.

The next figure, Fig. 2, shows another embryo that developed from an egg injured at the top with a hot needle during the two cell stage. The exovate appears to lie at one side, but was not attached, and fell off when the membranes were removed. Exovates of this sort that have shifted their position might easily lead to erroneous conclusions, if it were not known that they had become detached and had lost their place. A section of this egg[1]), Fig. *B*, shows that the injury has mainly involved the top of the posterior side of the interior rather than the surface. The in-pulling of the yolk cells, to form the gastrula rim, is seen on the left side of the figure. Other sections show a deeper invagination, and the opposite side of the egg is more injured than in this section.

Fig. 3 is also from an egg in which the top was stuck at the two-cell stage. An embryonic region is faintly outlined on the surface, and the sections show that the dorsal region of the embryo is present, beneath which there is a deep archenteron extending high up in the dark hemisphere, Fig. *C*. The opposite side of the egg is much injured.

Fig. 4 of the same set shows that a very small part of the egg has been left. Sections show, Fig. *D*, that while the nuclei have divided to a small extent the protoplasm is undivided and much vacuolated.

Fig. 5, also of the same series, shows an embryo in which a small blastopore is present. Near it, and attached to the egg, is a small exovate which is also gastrulating, Fig. *E*. It might be imagined that this exovate arose at the top of the egg and gastrulated along with the rest of the egg, but such an inference would be, I believe, erroneous. Either the injury involved the material on the posterior side of the egg above the equator, or else the posterior side of the egg has burst during the gastrulation period, and the ectoderm of the extruded part continued to roll over the yolk portion. Cases like this if not carefully followed, may easily lead to a wrong interpretation.

The following four cases represent embryos from eggs that were stuck at the top of the four-cell stage.

Fig. 6 represents an egg in which some of the top has been

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[1]) Right and left sides are reversed in the surface views and sections.

lost; a part of the material remaining sticking to the top. A faint gastrula-rim extends around the egg. Sections show, Fig. *F*, that much of the material of the top has been lost. A large segmentation cavity is present. Very little uninjured material is found above the blastoporic rim, and this shows scarcely any differentiation into ecto- and mesoderm.

The embryo drawn in Fig. 7 is similar in many ways to the last, but a deeper invagination has taken place, Fig. *G*. The differentiation anterior to the blastopore is much further carried out than in the last case. The top of the egg, and the whole of the posterior side is devoid of ectoderm.

Fig. 8 shows an embryo much reduced in size, having lost much of the material of the top and posterior sides. Gastrulation has nevertheless taken place, and in the region above the dorsal lip the layers are well differentiated, Fig. *H*.

Fig. 9 also represents an embryo much reduced in size, but with a large gastrula-mouth that extends well into the yolk, as shown in sections, Fig. *I*. Ectoderm and mesoderm are to be distinguished in the region above the invagination, but indications of an embryo are lacking.

Summary.

The results show that by removing the material of the top of the egg much of the embryo-forming-substance is lost, and although these eggs were kept for some time the dorsal parts of the embryo did not appear. The region of the blastoporic lip is usually left, and invagination takes place. The process of invagination is apparently independent of the presence of embryo-forming material anterior to the region of gastrulation. The results indicate, if they do not prove, that the material of the gray crescent is less often lost than the material of other parts of the egg, and it is not improbable that this may be due to its being more tenacious, so that it does not exude when the other substances flow out of the opening. A ring of similar substance must be supposed to exist around the egg, so that the blastoporic rim also often develops; but the material on the posterior side is more often lost than that of the ring elsewhere.

While the results support, in general, the conclusions reached in the VI paper of this series, yet taken alone they are too fragmentary to establish the above mentioned conclusions, and the operation itself is too uncertain to draw further inferences than those indicated above. Nevertheless it is certainly significant to find that when the material of the top of the egg is lost, the dorsal structures fail to put in an appearance, while the gastrulation process begins independently of their presence or absence.

Zusammenfassung.

Das Ergebnis lehrt, daß bei Entfernung des Materials von der Eispitze viel von der embryobildenden Substanz verloren geht, und obgleich diese Eier eine Zeitlang erhalten wurden, so erschienen doch die dorsalen Teile des Embryo nicht. Die Region der Blastoporuslippe bleibt gewöhnlich übrig und so findet Invagination statt. Der Invaginationsprozeß ist anscheinend unabhängig von dem Vorhandensein embryobildenden Materials nach vorn von der Gastrulationsstelle. Die Ergebnisse liefern, wenn keinen Beweis, so doch einen Hinweis, daß das Material des grauen Streifs weniger oft verloren geht als das Material andrer Eiteile und es ist nicht unwahrscheinlich, daß das von seiner größeren Zähigkeit kommt, vermöge deren es nicht exsudiert, wenn die andern Substanzen aus der Offnung ausfließen. Man muß die Existenz eines Ringes ähnlicher Substanz rund um das Ei herum annehmen, so daß sich auch die Blastoporusspalte oft entwickelt; aber das Material an der hinteren Seite geht öfter verloren, als das der übrigen Ringbezirke.

Während die Ergebnisse im allgemeinen die in der VI. Arbeit dieser Serie gezogenen Schlüsse stützen, sind sie doch, allein genommen, zu fragmentarisch, um die oben erwähnten Schlüsse allein aufzustellen, und die Operation selbst ist zu unsicher, um fernere Folgerungen als die oben angedeuteten daraus zu ziehen. Nichtsdestoweniger ist es gewiß sehr bezeichnend, daß nach dem Verluste des Eischeitelmaterials die dorsalen Strukturen nicht in Erscheinung treten, während der Gastrulationsprozeß unabhängig von ihrem Fehlen oder Vorhandensein anfängt.

The Relation Between Normal and Abnormal Development of the Embryo of the Frog:

VIII. As Determined by Injuries Caused by a Low Temperature.

By

T. H. Morgan.

With Plates XXI and XXII and 9 figures in text.

The only experiments on the frog's egg that deal directly with the injurious effects of cold on its development are those of HERTWIG ('94, '96, '98), SCHULTZE ('94, '99), LILLIE and KNOWLTON ('97) and my own ('91, '97, '99, '02).

HERTWIG fertilized the eggs of *Rana fusca* at 9° C. kept them at this temperature for two hours and then slowly (in one and a half hours) cooled them to 1.5° C. The early development was greatly prolonged, but no abnormal development took place. In another experiment the eggs were fertilized and then placed in cold water at 7°, then at 6°, and so on rapidly to 1.5° C. The eggs developed abnormally, and finally died. Even those kept at 2, 3, and 4° died. The differences in the two set of results was due, HERTWIG thinks, to the more gradual change in the former case. It seems to me, on the contrary, not improbable that the difference may have been due to the fertilization stages being affected by the cold in the latter experiment, and later stages (early segmentation) in the former, rather than the rate at which the eggs were cooled.

SCHULTZE found '95 that the eggs of *Rana fusca*, kept in the cold at a temperature near the freezing point for thirty days, ceased to develop, but when returned to higher temperatures proceed to develop normally. In a later paper, '99, SCHULTZE states that the temperature of freezing water does not entirely stop the development of the eggs of *Rana fusca*; although the process goes on very slowly. He found that if the eggs are kept at this temperature for less than thirty days, normal development may follow, but if kept longer the eggs may become abnormal. SCHULTZE's later paper seems to show that the difference between his own results and those of HERTWIG can not be entirely due to differences in the time at which the eggs were put into the cold, although this appears to be one of the principal causes of difference.

LILLIE and KNOWLTON found ('97) for the egg of *Rana virescens*, that below 4° C. the development is less normal; at 0° it comes to a standstill, at 1° the lower hemisphere fails to segment, although the segmentation continues in the upper hemisphere.

In '91 I pointed out that the eggs of *Rana sylvatica* which are laid early in the spring are often caught in the ice. Those eggs that are surrounded by the ice are often injured, but those that lie in the ice cold water beneath will continue to develop normally. The eggs of *Rana palustris* are laid later in the spring and if put into ice cold water during the fertilization period or even during the very early cleavage period they become injured and develop abnormally.

The material described in the present paper was collected at Bryn Mawr during the springs of '93 and '94. The eggs are those of *Rana palustris.* The eggs, usually in the two- or four-cell stages, were put into flat dishes containing water, which were placed on the bottom of an ice chest. The water from the melting ice covered for half an inch or less the bottom of the chest. The actual temperature of the water in which the eggs lay was found to be about one degree centigrade. Slightly above the bottom of the dishes the temperature ranged one or two degrees higher. From time to time, after two or three weeks as a rule, the eggs were taken from the ice chest and brought to room temperature where at intervals of a day apart they were preserved as the more interesting forms of abnormalities appeared.

Previous experiments had shown that the unsegmented but fertilized eggs of *Rana palustris* are much injured by temperatures as low as those employed in these experiments. The first few divisions may appear although irregularly, especially if placed on the ice soon after fertilization. Such eggs die without further development if kept longer in the cold. The blastula and gastrula stages stand the cold better, and may afterwards continue normal development. The young tadpoles may be kept six weeks or more without suffering permanent injury.

My intention was to study the different forms of abnormal development rather than the effects of a given temperature on each stage of development. In fact, in order to carry out a complete study of the latter problem it would be necessary to know not only whether an egg is in the two- or in the four-cell stage at the time it is subjected to the cold, but also the precise condition of the karyokinetic figure at the time of the experiment, since the recent results of Lyon and of Spalding have shown that the eggs of the sea urchin are more sensitive at certain phases of division than at others.

Results.

The embryos represented in Plate XXI Figs. 1—12 have been selected to show some of the more unusual forms that occur in the series.

The first drawing, Fig. 1, represents the anterior end of an embryo lying high up on the black hemisphere. The rim between the black and the white represents, in part at least, a blastoporic edge; although imperfectly developed on the side of the egg opposite to that shown in the figure. A longitudinal section through this embryo is drawn in Plate XXII Fig. *A*. On the left side of the figure the presence of three germ layers are seen in the anterior part of the embryo. A deep archenteric cavity is present. On the opposite side a slight turning-in is observed, that is high up on the egg. It represents the blastoporic rim. On this side there is very little differentiation into germ layers. A large segmentation cavity is present whose sides are filled in but whose concave bottom extends down into the yolk. In the normal embryo, at the time of gastrulation, the bottom of the segmentation cavity is convex. If this convexity results from the pushing upwards of the yolk it would allow the rolling downwards of the sides when the blastoporic rim is forming. The lower portion of the yolk in this embryo appears to have been somewhat injured.

The next figure, Fig. 2, also represents the anterior end of an embryo more fully developed than the last. Sections show no trace of a segmentation cavity, which, if present in an early stage, has been obliterated by the growing forward of the anterior end of the embryo. A deep archenteron extends forward from the posterior edge under the embryo. The blastoporic rim on the opposite side of the egg is scarcely developed. The yolk at the bottom of the white hemisphere is killed; the rest of it is divided up into cells.

The next embryo, Fig. 3, also represents the anterior end of an embryo which appears to extend backwards over the yolk. A cross-section of this embryo is drawn in Fig. *B*. The sides are injured and the ectoderm is thickeded and its cells much crowded. There is no indication of a blastoporic rim at the sides where the material is injured. The medullary plate, notochord, mesoderm, and archenteron are seen in the figure. The yolk is much injured. There is no evidence to show that the posterior end of the embryo has actually grown backwards. It appears rather as though the uninjured material at the top of the egg has drawn together along the median plane, and an embryo has formed in situ. Had the posterior end extended backwards, the sides would be expected to show, as in other cases where backgrowth has taken place, an inturning of the edges.

Figs. 4 and 5 show the opposite sides of an embryo in which also only the anterior end has developed. A longitudinal section of this embryo is shown in Fig. *C*. The top of the egg is solid. The archenteron divides anteriorly, one branch probably representing the liver. The opposite side of the egg shows scarcely any differentiation into a blastoporic rim, although the mesoderm has developed as far as the black-white edge. The lower parts of the yolk are injured, although not very much so. On one side a large cavity is present, which is shown in other sections.

Fig. 6 shows an egg in which the embryo has not appeared. This is, in fact, the condition found in the majority of eggs. A section through this egg is shown in Fig. *D*, in which, the whole top of the egg has drawn together into a solid mass, the greater part ot which appears to be ectoderm with some mesoderm in the middle. The yolk is almost completely destroyed.

The next egg, Fig. 7, is similar, but contains a large vacuole in the lower part of the yolk. Sections, Fig. *E*, show that the upper part of the egg is a dense mass in which the different layers can not be sharply distinguished. Another egg like the last one (not drawn) shows in section the conditions represented in Fig. *F*. Here the edge is turned in, but there is no certain indication that this inturning represents an attempt at gastrulation. Ectoderm covers the surface, mesoderm lies beneath this, and in the middle and lower portions some yolk cells are present. The yolk portion of the egg is dead, or at least unsegmented.

The five remaining figures represent a type of development that presents, in certain respects, a contrast with the preceeding types.

Fig. 8 shows a typical ring-embryo. The two sides of the embryo encircle the yolk, and the yolk exposure extends forward to the anterior end of the medullary plate. In this latter respect especially, this type of embryo differs from the preceeding ones. Moreover the difference is not due in the former case to the greater advance of the dorsal lip of the blastopore; for, in both the dorsal lip remains largely stationary. The difference consists rather in the concentration and downgrowth of the material that goes to form the head anterior to the dorsal lip in the former cases, and to the failure of the material of the early embryo to extend downwards in the latter. Cross-sections (not figured) of this embryo shows that the bottom of the yolk was injured. There is a very large vacuole in the center of the yolk mass, which is not the segmentation cavity. On each side, the medullary plate, notochord, and mesoderm extend entirely around the rim. An archenteric invagination is absent, except just at the anterior end, where it is better developed on one side than the other.

The next figure, Fig. 9, represents a half-embryo that is more complete anteriorly than in the middle and posterior regions. Cross-sections confirm this interpretation. A cross-section through the head is shown in Fig. *G*, where nearly a complete medullary plate is present, although there is a noticeable lack of mesoderm on the less developed side. This absence of mesoderm has, I think, an important signification, as I shall point out later. A section through the middle of this embryo is shown in Fig. *H*. Here the half-condition is more evident. The notochord, however, does not lie quite at the edge of the yolk, as there is a small mass of mesoderm between it and the yolk. It is important also to notice that there is no blastoporic rim on the opposite side, but the ectoderm ends abruptly at the injured portion of the yolk. Nevertheless this edge is to be thought of as an undeveloped blastoporic rim, and the embryo represents, from this point of view, a ring-embryo with one side undeveloped. Certain limitations must be made to this statement in regard to the head region, but this will be discussed later.

The next embryo, Fig. 10, is also a half-embryo. The anterior end of the head is, however, a whole structure, although somewhat reduced in size, Fig. *I*. A cross-section through the middle of this embryo, Fig. *J*, shows the half medullary fold, an archenteric invagination, and mesoderm. Another half-embryo (not drawn), resembling the last in most respects, was also cut into cross-sections, Fig. *K* (by an oversight the notochord was omitted in the drawing; it lies in the same position as in Fig. *J*).

The next embryo, Fig. 11, appears in surface view to be more nearly a whole embryo, lying along one side of the yolk. The cross-section — although somewhat defective — show that while the anterior end is more nearly a whole structure, posteriorly, as shown in Fig. *L*, the embryo appears more nearly a half, although the position of the medullarly groove lying a little inwards from the edge gives the appearance of a whole plate. The opposite side of the embryo is quite undeveloped.

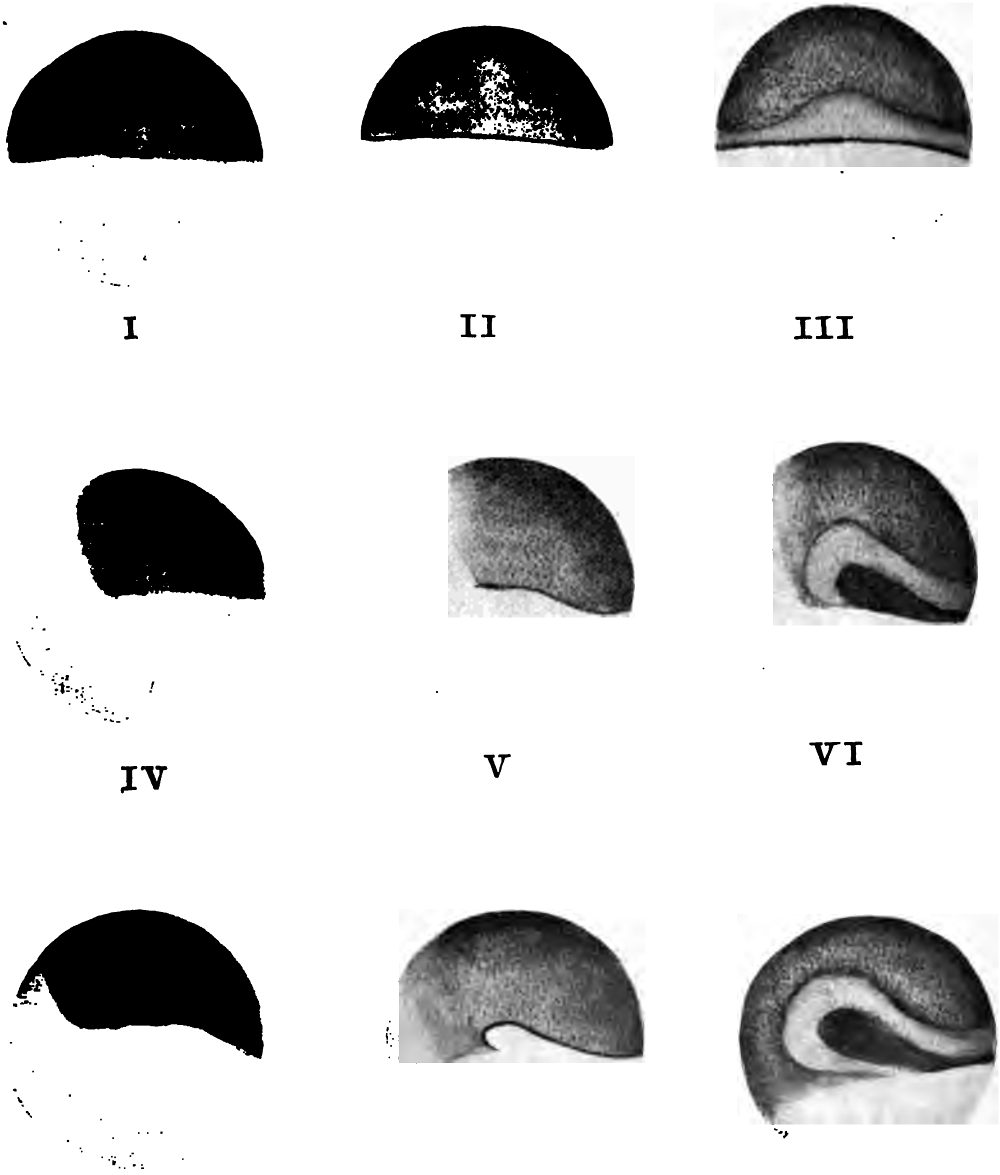
Finally the last embryo, Fig. 12, appears, in surface view, much more certainly a whole embryo with the yolk lying at one side. The three sections of this embryo, Figs. *M*, *N*, *O*, show that this is the correct interpretation. The head region is symmetrically developed, Fig. *M*, and closed all round. Just back of this part the embryo is defective on one side, Fig. *N*, but the medullary plate is still nearly a whole structure. The notochord lies beneath its middle and mesoderm is present on each side of the notochord. Further back, Fig. *O*, the medullary plate loses more and more its whole structure, and the mesoderm on the injured side decreases in amount until finally near the posterior end little more than a half structure is present. The other free edge of ectoderm (to the right and below in the sections) can scarcely be identified as a blastoporic rim, and probably does not now represent such a structure.

The interpretation of the last four embryos, which appear to form a more or less continuous series, seems to me to be as follows. The embryo-forming material of the upper hemisphere is more injured on one side than on the other, and from the more perfect side the half-embryo develops. If some of the material of the injured side is carried backwards along with the dorsal lip of the uninjured side it will tend to make the embryo more complete on this side. The decreasing amount of this material from before backwards is explicable on this hypothesis. That this side should be defective in amount of material is accounted for on the assumption that only part of the embryo-forming material situated anterior and lateral to the dorsal lip of the blastopore is available. The rest of the material that lies farther out at the side has been injured, or is too far away to be drawn into the dorsal lip.

By means of the following diagrams I have attempted to make clearer my interpretation of the development of the embryos resulting from the effects of cold. In the first line of figures, I, II, III, the yolk hemisphere alone is represented as injured, and the embryo-forming material differentiates in situ above the equator of the egg, and produces the ring-embryo type. In the second three diagrams, IV, V, VI, the yolk and part of the embryo-forming-regions of one side have been injured, and a half-embryo results, whose head may be more than a half structure, either because a whole head tends to develop out of the undivided material in front of the dorsal lip (as in Roux's half-embryos), or because somewhat more than half of the material is present in the region where the head forms. In the third line of figures, VII, VIII, IX, less of the upper parts of one side of the egg is injured, and, in consequence, some of the material grows backwards along with the backgrowth of the dorsal lip of the blastopore to produce some of the material of the defective side. Hence more than a half-embryo appears on one side, especially anteriorly.

The twelve embryos described above given perhaps an exaggerated idea of the effects of cold on the development, because these are for the most part extreme forms that have been picked out to

Figs. I—IX.



I II III

IV V VI

VII VIII IX

illustrate the more interesting types that occur. Looking over the whole material the following general impression is obtained. Probably about 95 per cent of the eggs do not development beyond stages like those represented in Figs. 6 and 7, in which the upper hemisphere of dark cells is rather sharply separated from the yolk portion, and in which the embryo does not appear. In many of these the blastoporic rim does not develop, although it is not uncommon for an inturning more or less extensive to take place. A condition of spina bifida is frequently met with amongst the remaining forms, and this is sometimes combined with a shortening of the dorsal region producing embryos superficially similar to those described by TODD after injury to the blastopore region. Anterior embryos, like those drawn in Figs. 1 and 2, come next in frequency, then half-embryos, and most rarely of all embryos like that of Fig. 12, which has both sides present (one more incomplete) and the yolk at the side.

Summary.

1) Despite the fact that the ovarian eggs must be subjected throughout the winter to a temperature of freezing water they are not injured, although eggs in the fertilization stages and in the early cleavage stages are injured by prolonged immersion in cold water. The later stages of development again are less injured by the cold.

2) If the eggs of *Rana palustris*, in the two and four-cell stages, are kept for several weeks at a temperature of 1° C. they are injured, and develop, when returned to room temperature, abnormally. The lower hemisphere is more injured than the upper, as previous writers have observed.

3) If the lower hemisphere and the lower parts of the upper hemisphere are injured a small cap of black cells is formed, Figs. 5, 6, 7, which often turns under around its border to produce a blastoporic rim.

4) The anterior regions of the egg are often less injured than the rest and the anterior end of the embryo develops, Figs. 1, 2, 4. The head may also receive contributions from the material at the sides of the upper regions, which, drawing together, add to the length of the head region that is produced, Figs. 2, 3.

5) Ring-embryos are sometimes produced, in which the medullary plate may be split quite to its anterior end. In this case the material

of the sides differentiates in situ, and has not grown downwards as in the last case.

6) If one side of the egg is more injured than the other a half-embryo develops; Figs. 8, 9, 10, in which the head is usually something more than a half-structure.

7) If the anterior region of one side is less injured some of it may be drawn backwards with the advancing dorsal lip and produce a portion of the defective side; Fig. 12. In this case the yolk appears to lie at the side of the embryo.

8) In general, the effect of the cold is to injure parts of the egg, the rest of the egg then proceeding to form its part-structures. The injury to the lower hemisphere interferes with the pushing downwards of the materials of the top of the egg, and also with the lateral overgrowth of the lateral lips. In consequence the developing parts of the embryo appear higher up on the egg.

9) The results show that when a part of the egg of the frog is injured the remaining parts do not produce a whole structure of smaller size, but parts only of the embryo, and those parts that the same material would produce in the whole embryo. This does not necessarily mean that the material may not be totipotent within the limits of the layers, but that the relation of the parts to other structures or even the arrangement of the materials themselves may be such that only a part can, under the conditions, be developed.

Zusammenfassung.

1) Trotz der Tatsache, daß das Ovarialei den Winter hindurch der Gefriertemperatur des Wassers ausgesetzt sein muß, werden sie dadurch doch nicht geschädigt, obgleich Eier im Befruchtungs- und frühen Furchungsstadium durch längeren Aufenthalt in kaltem Wasser geschädigt werden. Die späteren Entwicklungsstadien erfahren wieder geringere Schädigung durch die Kälte.

2) Für mehrere Wochen bei einer Temperatur von 1° C. gehaltene Eier von *R. palustris* im Zwei- und Vierzellenstadium erfahren eine Schädigung und entwickeln sich nach der Rückkehr in Zimmertemperatur abnorm. Wie frühere Beobachter angeben, wird die untere Hälfte mehr geschädigt als die obere.

3) Wenn die untere Hälfte und die unteren Teile der oberen geschädigt wurden, so bildete sich eine kleine Mütze von schwarzen Zellen, Fig. 5, 6, 7, welche sich öfter entlang ihrem Rande zur Bildung einer Blastoporusspalte einkrümmt.

4) Die vorderen Eibezirke sind oft weniger geschädigt als der Rest und das Vorderende des Embryo gelangt zur Entwicklung, Fig. 1, 2, 4. Die Kopfanlage kann auch eine Beisteuer von dem seitlichen Material der oberen Bezirke erhalten, welches, sich zusammenziehend, zur Länge der gebildeten Kopfregion beiträgt, Fig. 2, 3.

5) Manchmal entstehen Ringembryonen, bei denen die Medullarplatte ganz bis an ihr vorderes Ende gespalten sein kann. In diesem Falle differenziert sich das seitliche Material in situ und dringt nicht, wie im vorigen Falle, nach unten vor.

6) Bei stärkerer Verletzung einer Eiseite entwickelt sich ein Halbembryo, Fig. 8, 9, 10, in welchem der Kopf gewöhnlich mehr als eine Halbbildung darstellt.

7) Wenn der vordere Bezirk der einen Seite weniger beeinträchtigt wurde, so kann er mit dem Vorschreiten der dorsalen Lippe nach hinten gezogen werden und einen Teil der defekten Seite bilden, Fig. 12. In diesem Falle scheint der Dotter an der Seite des Embryo zu liegen.

8) Im allgemeinen ist der Effekt der Kälte eine Verletzung nur von Eiteilen. Der Rest des Eies fährt dann fort, seine Teilbildungen zu schaffen. Die Schädigung der unteren Hälfte stört das Vorrücken des Eischeitelmaterials nach unten und auch das seitliche Überwachsen der lateralen Lippen. Infolgedessen erscheinen die sich entwickelnden Embryoteile höher oben am Ei.

9) Die Ergebnisse zeigen, daß nach Verletzung eines Teils beim Froschei der Rest nicht eine Ganzbildung von geringerer Größe hervorbringt, sondern nur Embryoteile, und zwar diejenigen, welche dasselbe Material im ganzen Embryo hervorbringen würde. Das bedeutet nicht notwendigerweise, daß das Material in den Grenzen der Keimblätter nicht totipotent ist, sondern daß die Beziehung der Teile zu andern Strukturen, oder sogar die Verteilung des Materials selbst derartig ist, daß unter den Bedingungen lediglich ein Teil sich entwickeln kann.

Literature.

Hertwig, O., Über den Einfluß äußerer Bedingungen auf die Entwickelung des Froscheies. Sitzungsber. d. Kgl. Preuß. Akad. d. Wiss. Phys.-math. Abth. 1894.

—— Über den Einfluß verschiedener Temperaturen auf die Entwickelung der Froscheier. Ibid. 1896.

—— Über den Einfluß der Temperatur auf die Entwickelung von Rana fusca und Rana esculenta. Arch. f. mikr. Anat. LI. 1898.

Lillie, F. R., and Knowlton, E. P., On the Effects of Temperature on the Development of Animals. Zool. Bull. I. 1897.

Morgan, T. H., Some Notes on the Breeding Habits and Embryology of Frogs. American Naturalist. 1891. p. 758.

—— The Development of the Frog's Egg. New York 1897. p. 168.

—— Regeneration of Tissue Composed of Parts of Two Species. Biol. Bull. I. 1899. p. 9 and 10.

—— The Relation Between Normal and Abnormal Development. I. 1902. p. 269.

Schultze, O., Über die Einwirkung niederer Temperatur auf die Entwickelung des Frosches. Anat. Anz. X. 1894.

—— Über die Einwirkung niederer Temperatur auf die Entwickelung des Frosches. II. Anat. Anz. XVI. 1899.

The Relation Between Normal and Abnormal Development of the Embryo of the Frog:

IX. As Determined by Insufficient Aeration.

By

T. H. Morgan.

With 18 figures in text.

The retarded development of eggs that lie in the middle of the bunch, or, if the bunch is confined in a flat dish, in the middle of the lower surface of the bunch, must have been observed by everyone who has kept frog's eggs in a confined space. The delay is caused probably in part by insufficient aeration, but whether because of lack of oxygen, or because of an excess of carbon dioxide or of some other substance excreted has not been sufficiently determined. Several conditions may often be involved, for, it has been shown by GODLEWSKI that carbon dioxide acts injuriously on the eggs of the frog, and SAMASSA, SCHULTZE, and GODLEWSKI have shown that an insufficient supply of oxygen also causes the egg to develop abnormally.

In the following pages I shall describe some abnormal embryos of *Rana palustris* that were taken from the under side of bunches of eggs developing in flat dishes. The embryos at the top of the bunch had begun to hatch when the oldest of these abnormal forms were preserved.

The most common feature, shown by all of the eggs that result from insufficient aeration, is that the yolk fails to be drawn under the black cap and the latter is generally sharply marked off from the yolk, often by an inturned edge. Ring-embryos are not infrequently found in lots of this sort, and some forms that originated in this way I have already described. I shall omit, therefore, an account of the ring-embryos and spina bifida forms in the present paper.

A not uncommon type is represented in text-figure 1 in which a dark hemisphere is separated from a white hemisphere by a narrower portion. A section through this embryo in the plane of the paper is shown in text-figure *A*. The black cap is made up of a thick layer of ectoderm. Around the sides it turns under to form a cavity. At the top of this cavity elongated cells are present that recall the elongated cells at the top of the archenteric invagination in the normal gastrula. The interior cells above this cavity may possibly correspond to mesoderm cells. The rest of the egg is composed of yolk cells in the middle of which there is a large cavity that extends from the top to the bottom of the egg. This cavity has the appearance of being an enlargement of the segmentation cavity, but an unbroken series of stages would be necessary to establish this point. It should, however, be recalled in this connection that in the late stages of normal development, after the yolk has grown upwards around the inside of the segmentation cavity, the latter sinks down into the yolk, and may come to lie close to the floor of the archenteron, being separated from it by only a single line of cells that is often broken in preserving or in cutting the egg. The floor of the segmentation cavity is derived from the cells of the lower hemisphere, so that the extension downwards of the segmentation cavity in these abnormal embryos may not be so very different from the changes that take place in the normal. The flattening of many of the cells that border the central cavity is noticeable but the meaning of this is obscure.

An embryo similar in many ways to the last is shown in Fig. 2. The conditions are similar to the last, except for the much smaller central cavity, which is largely confined to the bottom of the egg, although there is a central core of loose cells through the middle of the embryo.

The next two embryos, Figs. 3 and 4, are much larger than the preceding ones. The enlargement is clearly associated with an enormous cavity in the interior of the egg, as shown in Figs. *C*, *D*. These eggs are so abnormal and so little developed that there is little hope of identifying the different parts. There is no sharp line between the dark and the light cells, but the deeper pigmentation of a band of cells near the equator may indicate the region of the blastoporic rim of less modified eggs[1]).

Another embryo, externally much like the preceding, is shown in surface view in Fig. 5, and in section in Fig. *E*. In this embryo a large part of the yolk has been so seriously injured that it has failed to become cellulated (or if it divided in the early stages the planes of division have disappeared). On one side an archenteron

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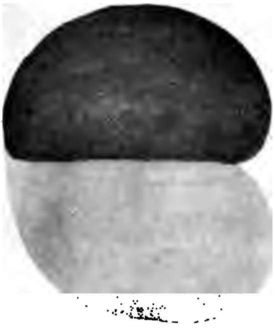
[1]) I have found somewhat similar embryos from eggs kept in the cold, but here too the influence may have been due to lack of aeration rather than to the cold.

has developed, and here above the archenteron and around its top a few yolk cells are present. A large segmentation cavity occupies the top and middle of the yolk. It is probable that the dorsal lip of the blastopore has pushed backwards for a short distance over the yolk. A small amount of mesoderm also appears in the lip of the blastopore.

Fig. 6 shows the anterior end of an embryo lying apparently high up on the dark hemisphere. The longitudinal section Fig. *F* gives a more correct idea of the conditions. The section shows that half, at least, of the top is dead, and has lost much of its pigment. Hence the embryo appears to lie nearer the top of the egg than is really the case, since, with the loss of pigment on one side, the top is no longer so sharply defined. Practically all of the yolk is dead and vacuolated. It appears, therefore, that only one side of the egg has remained alive, and has formed the anterior end of the embryo; in part by a backgrowth over the dead yolk, which must have been brought about, no doubt, largely by the approach of the lateral lips to the middle line. The archenteron is a long slit with its anterior walls surrounded by the few yolk cells that are present. The roof of the remaining part is covered by small cells, which we must suppose to have been carried to this region by the coming together of the sides of the blastopore in the dorsal lip. As this occured the posterior end extended over the yolk. Thus while the embryo appears, in surface view, to lie over the top of the egg, it occupies in reality one side. It may be admitted as a possibility that as the lateral lips approach the anterior end of the embryo may as a whole be shoved somewhat forward, so that as it forms the head-end may be carried to a position a little anterior to that at which it first appeared.

The next figure, Fig. 7, shows the anterior end of an embryo with the medullary plate in process of rolling in. At its posterior end the plate appears as though split into right and left halves between which the yolk appears on the surface as a V-shaped mass at the posterior end. A longitudinal median section, Fig. *G*, shows that this type of abnormality has been caused by injury to the yolk of the lower hemisphere, so that it fails to be carried under the dorsal and lateral lips of the blastopore. The latter have advanced only for a short distance at the sides. The section was a little oblique so that the medullary plate is not cut through its entire length. The ventral lip has failed completely to develop.

Figs. 1—9.



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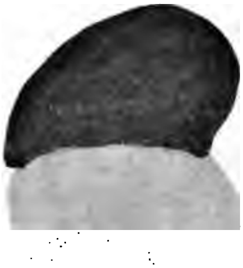

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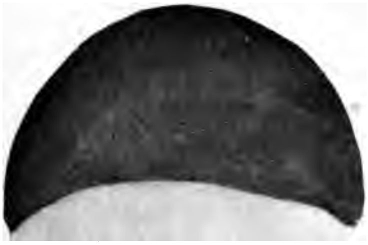

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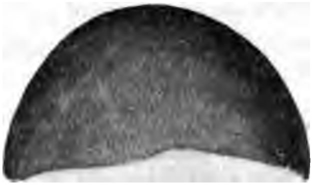

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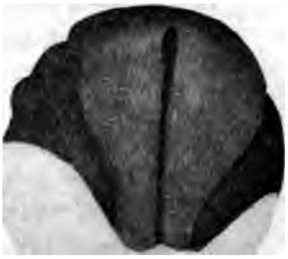

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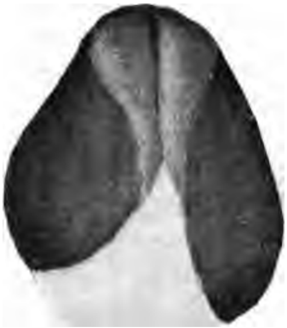

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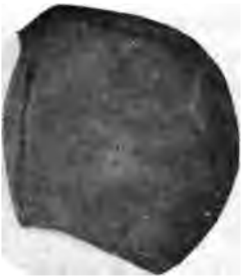

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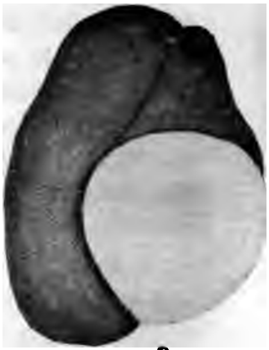

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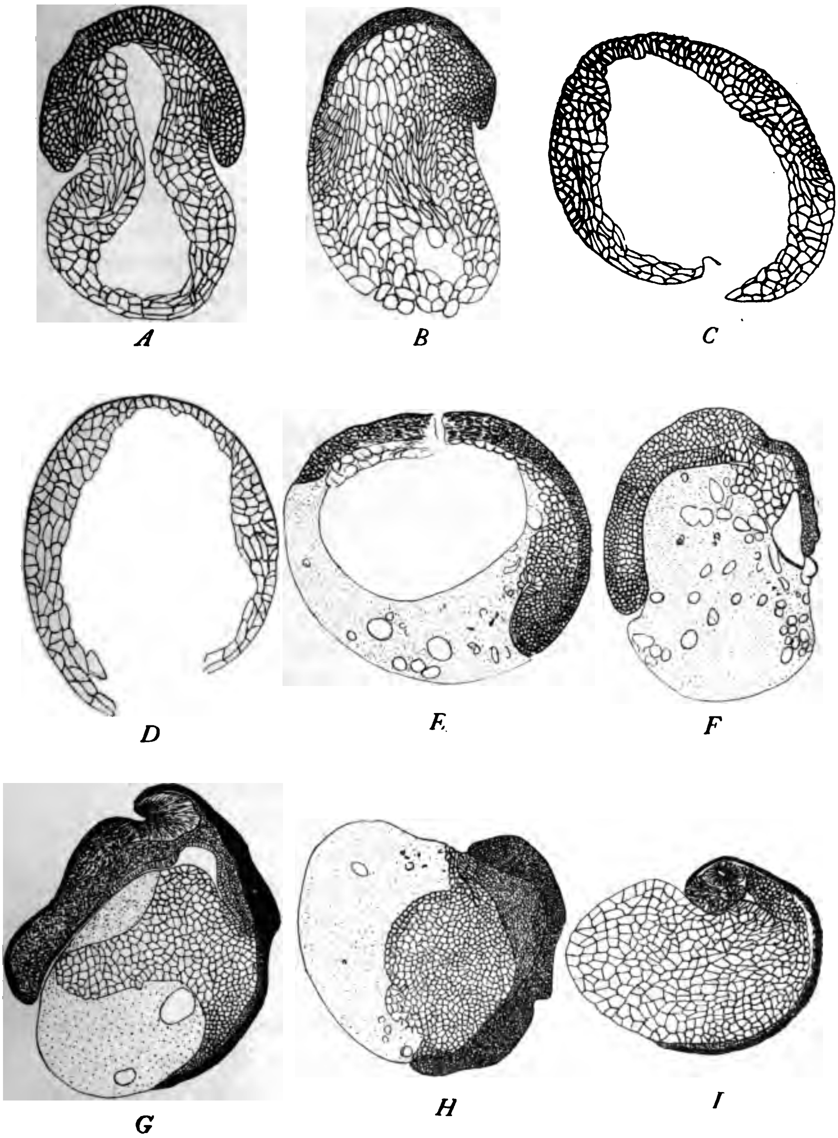
The embryo drawn in Fig. 8 shows, in surface view, little more than a black and a white half, sharply separated by an inturned rim. In section, Fig. *H*, an imperfectly formed half-embryo is found along the black white edge. The medullary plate is scarcely developed;

Figs. *A—I.*



a notochord lies below or near the edge, with an archenteric slit on its inner side. The other half of the egg is so much injured that it has failed to produce an embryo.

An embryo with an exposure of yolk at the side is shown in Fig. 9. The head is nearly normal and equally developed on both sides, but the middle and posterior regions of the embryo are incomplete on the side of the exposed yolk. A cross-section through this region is represented in Fig. *I*. The dorsal side appears more than a half structure, at least to the extent of having some mesoderm on the inner side of the notochord. The inturning of the archenteron, just outside of this small patch of mesoderm, shows that the region represents the blastoporic rim of the right side of the embryo. The mesoderm may be looked upon as having arisen as an extension backwards of the mesoderm of the right side from the more anterior regions of the embryo that are symmetrically developed, or it may be supposed to be due to the notochord developing a little inside of the edge, so that some of the mesoderm is left on the yolk side. Both interpretations, in the light of results of this sort in other cases, seems to me to be the more possible. The section shows also the other side of the exposed yolk (at the bottom of the figure), which theoretically corresponds to the missing half of the blastoporic rim. No development has taken place on this side, and it is due to this that the yolk has remained uncovered. It is worthy of notice, that here, as in other cases of this sort in which one blastoporic rim fails to develop, the developing side produces something more than half.

Summary.

1) The principal injury caused by insufficient aeration is to the yolk which failing to turn in interferes with the downgrowth of the material at the equator of the egg. Ring embryos and embryos with spina bifida are of frequent occurence.

2) More extreme injury results when the earlier stages are affected producing embryos like those shown in Figs. 1 (*A*), 2 (*B*), 3 (*C*), 4 (*D*). In these the segmentation cavity has entended downwards into the yolk instead of outwards towards the sides.

3) In other cases the yolk may be actually so injured that it fails to segment, or, if segmented, the cells walls disappear and the yolk becomes vacuolated or dead. According to the extent of injury

to the yolk different types of embryos appear, some of which are shown in Figs. 5 (*E*), 6 (*F*), 7 (*G*). In most cases the injury involves equally all sides of the yolk so that a circular blastoporic rim develops Figs. 5—7.

In other cases, Figs. 8 and 9, one side may be more injured than another so that it develops less. This difference may be due to one side of the egg being in a better position to become aerated than the other.

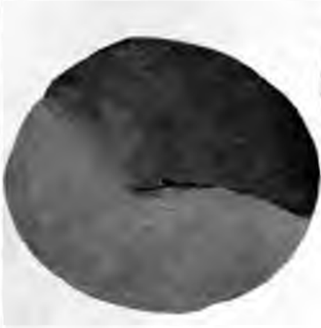
Zusammenfassung.

1) Die Schädigung, welche ungenügende Durchlüftung prinzipiell hervorbringt, geht den Dotter an, welcher aus Mangel an Einwärtsbewegung das Herabwachsen des Materials vom Eiäquator stört. Ringembryonen und solche mit Spina bifida kommen häufig vor.

2) Extremere Schädigung ergibt sich, wenn die früheren Stadien betroffen werden; es entstehen dann Embryonen ähnlich denen in Fig. 1 (*A*), 2 (*B*), 3 (*C*), 4 (*D*). Bei diesen hat sich die Furchungshöhle nach unten in den Dotter ausgedehnt, anstatt nach außen, nach den Seiten zu.

3) In andern Fällen kann der Dotter tatsächlich so verletzt sein, daß seine Furchung ausfällt, oder daß im Falle der Furchung die Zellwände verschwinden und der Dotter vacuolisiert wird oder abstirbt. Entsprechend der Ausdehnung der Dotterschädigung erscheinen verschiedene Embryotypen, von denen einige in Fig. 5 (*E*), 6 (*F*), 7 (*G*) dargestellt sind. Dabei bringt in den meisten Fällen die Schädigung den Dotter auf allen Seiten in der Entwickelung zurück, so daß sich eine zirkuläre Blastoporusspalte entwickelt (Fig. 5—7).

In andern Fällen (Fig. 8 und 9) kann eine Seite mehr geschädigt sein als eine andre, so daß die letztere sich besser entwickelt. Diese Verschiedenheit kann von einer günstigeren Lage der einen Eiseite mit bezug auf die Durchlüftung bedingt sein.



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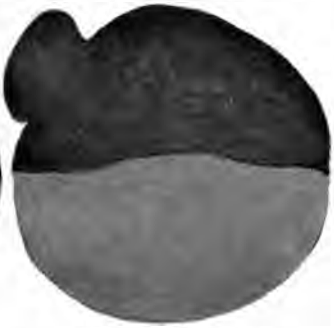

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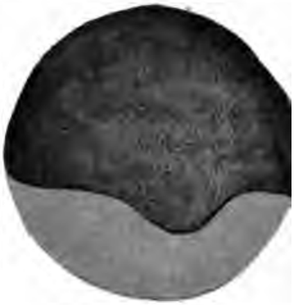

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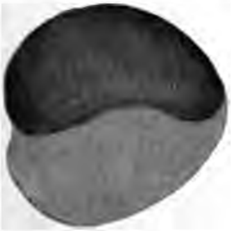

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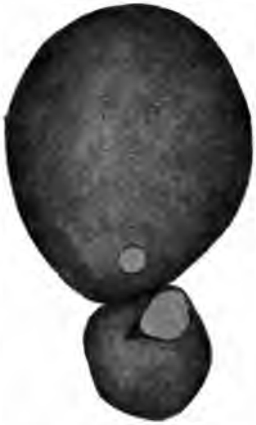

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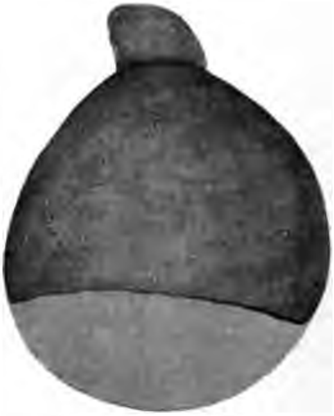

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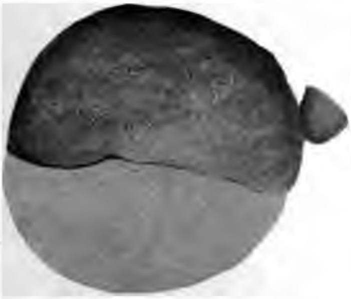

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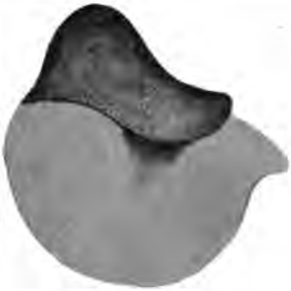

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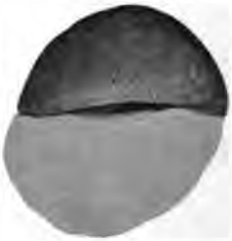

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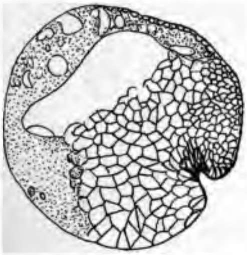

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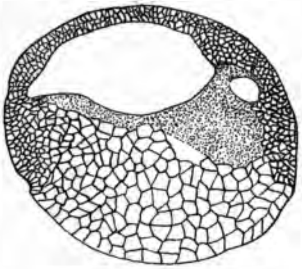

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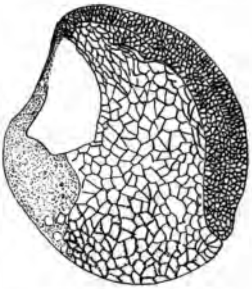

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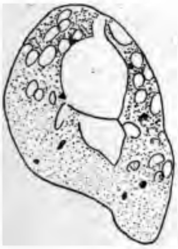

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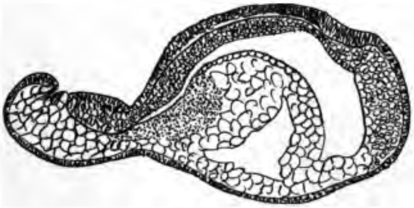

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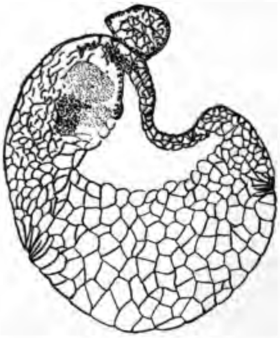

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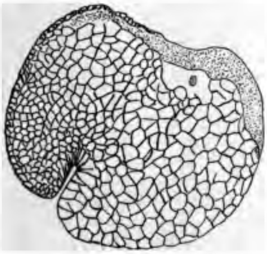

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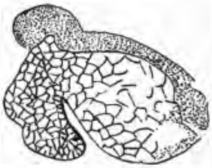

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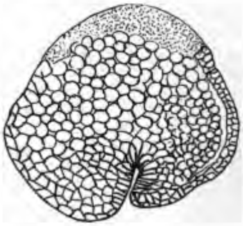

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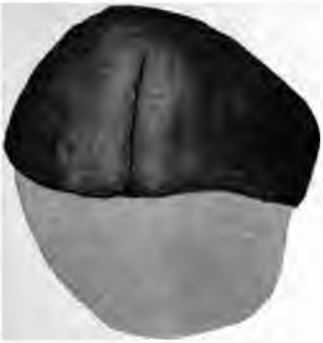

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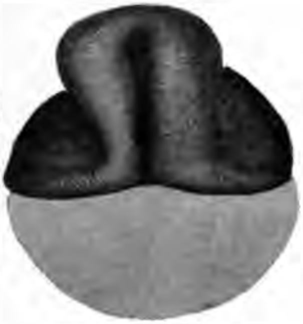

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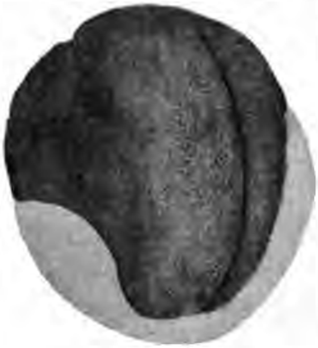

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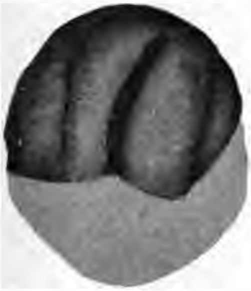

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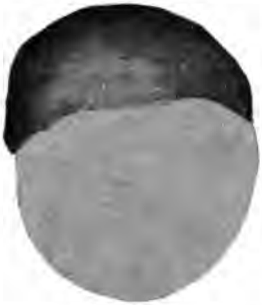

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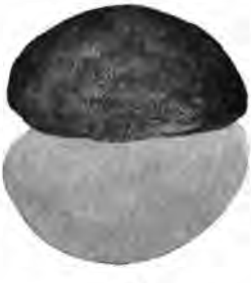

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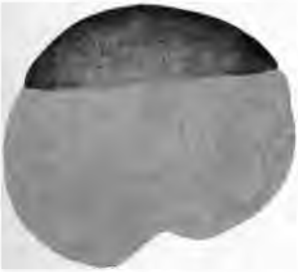

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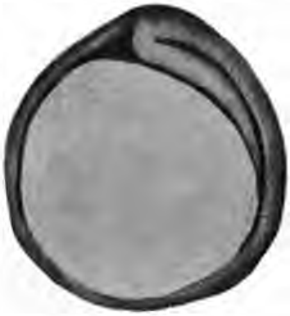

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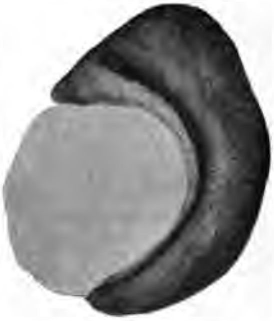

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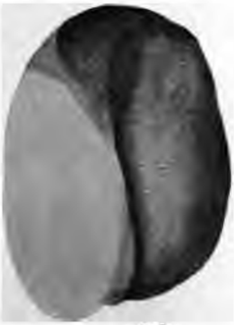

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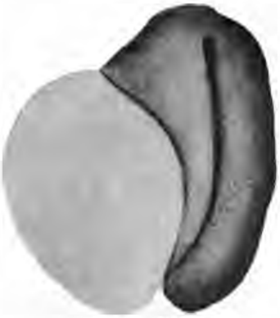

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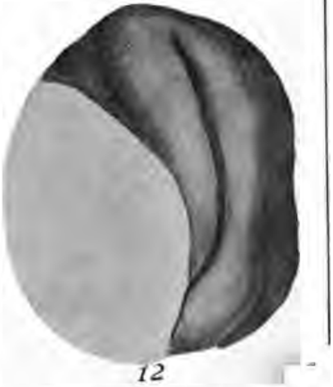

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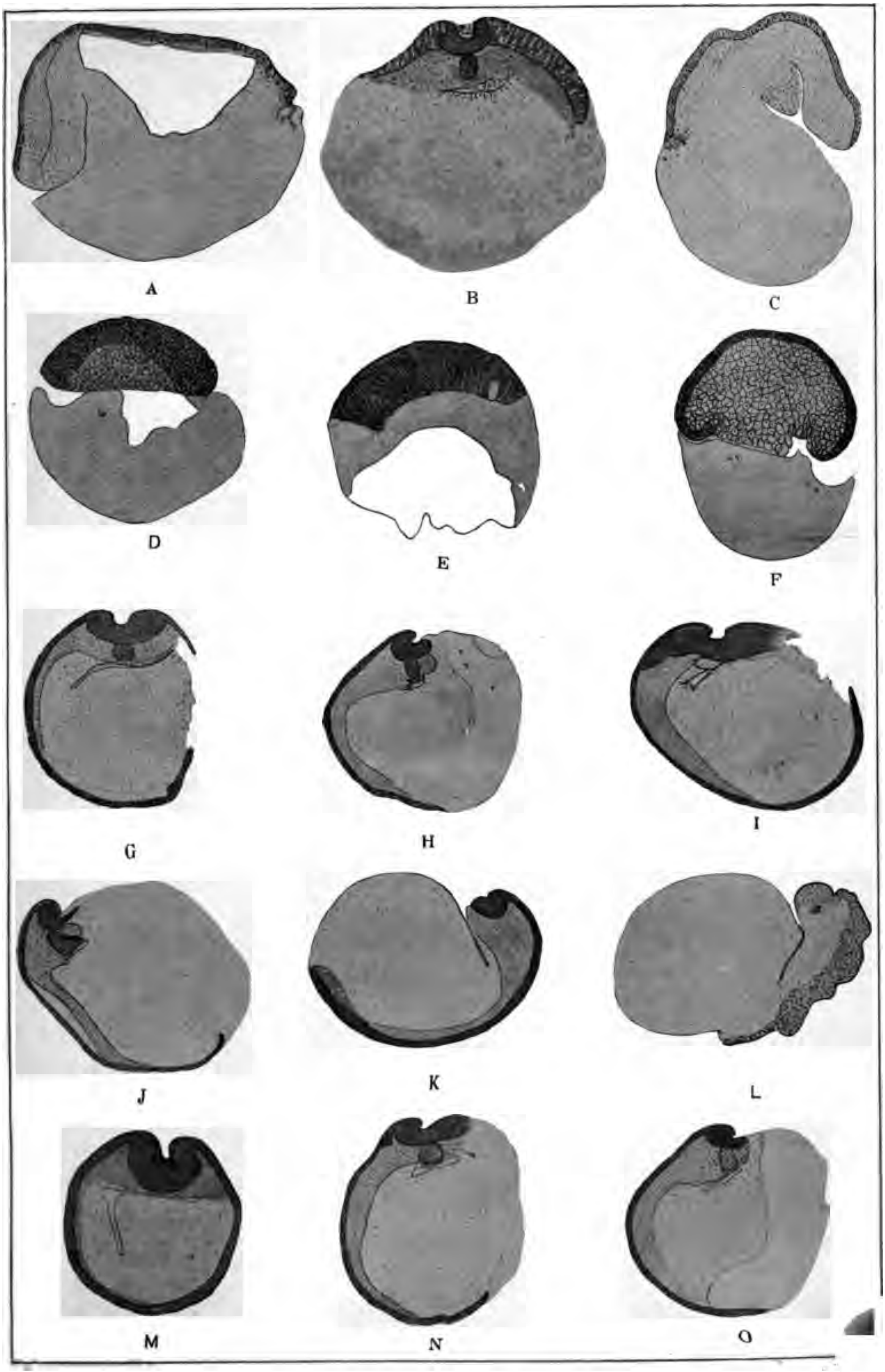

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The Relation Between Normal and Abnormal Development of the Embryo of the Frog:

X. A Re-examination of the Early Stages of Normal Development from the Point of View of the Results of Abnormal Development.

By

T. H. Morgan.

With 23 figures in text.

Eingegangen am 13. März 1905.

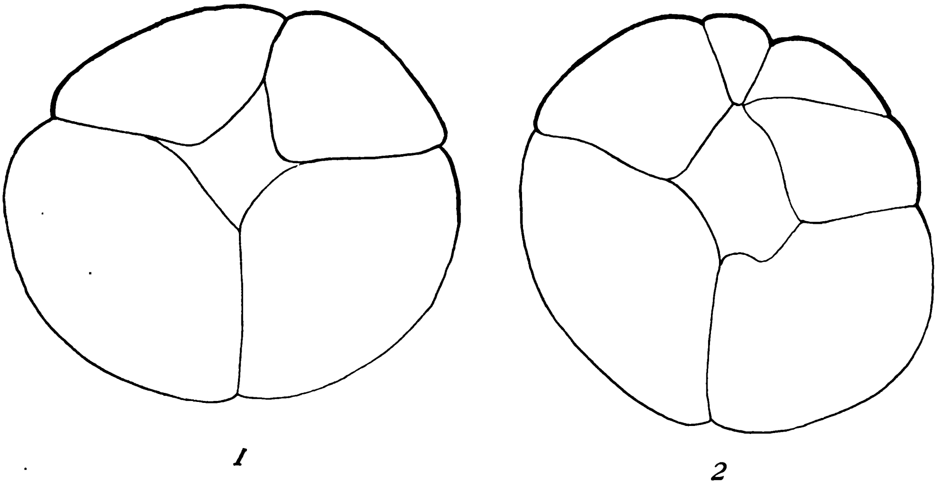
The preceding studies, I—IX, on the influence of external agents on development have shown that the same general types of abnormalities appear over and over again independently of the agent employed. One of the most striking results is the frequent appearance of an abnormal embryo in the upper hemisphere of the egg, although in the normal development the material out of which the embryo develops is supposed by most recent writers to come from the lower hemisphere. This difference in the two cases suggested that some, at least, of the embryo-forming material comes from the upper hemisphere, and this led me to carry out the experiment of destroying the upper four cells with the results described in the Vth paper of this series. It was found that parts of the embryo failed, under these circumstances, to appear.

The development of certain types of abnormal embryos in the upper hemisphere, and the development of the normal embryo below the equator of the egg, might be harmonized in either of two ways; — either the material that produces the abnormal embryo is different from that which goes to form the normal, or else there is a down-wandering of material of the upper hemisphere at a stage earlier than has, heretofore, been suspected. It seemed that this latter possibility might be tested by a re-examination of the normal development during the cleavage, blastula, and gastrulata stages; for, despite the fact that these stages have been so often described, a renewed study has convinced me that some of the most essential changes occuring during the early development have been overlooked. In a word, a study of the early stages has shown that normally a large part of the material of the upper hemisphere is transferred downwards during the segmentation and early gastrula stages, and that this is the material out of which the dorsal parts of the embryo (the »embryo« in a narrower sense) develops. The failure of this material to be carried downwards in many forms of abnormal development accounts for the appearance of the embryo on the top of the egg. The evidence on which this statement rests will be given in detail in the following pages.

The Cleavage Period and the Enlargement of the Segmentation Cavity.

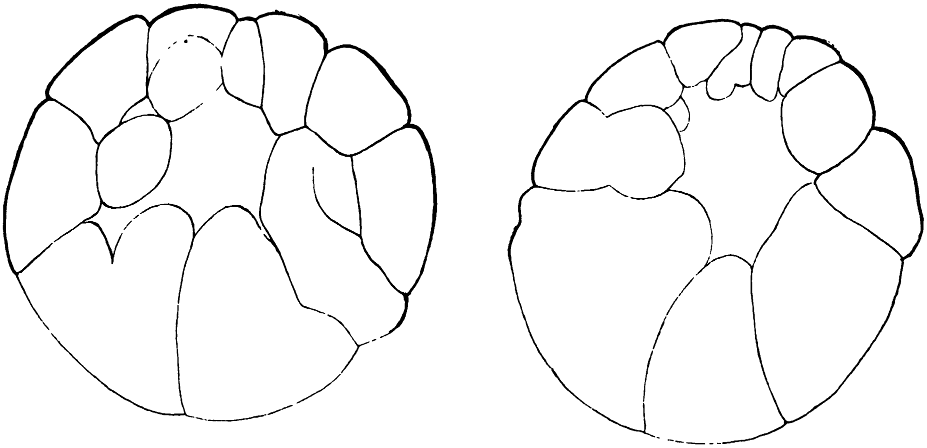
The segmentation cavity appears as a conspicuous space in the upper hemisphere at the eight-cell stage. As shown in Fig. 1 the cavity is at first confined largely to the upper hemisphere, although its lower end may extend below the equator. A 16-cell stage is shown in Fig. 2. During the following cleavage-stages, 32-, 64-cell stage, etc., the segmentation cavity may have very different outlines in different eggs, and at different times. Nevertheless it tends to become larger and to broaden out laterally as development proceeds. It is during this period that some of the surface cells of the upper

Figs. 1 and 2.



hemisphere begin to pull into the interior, as **Reed** has recently shown. The following figures show sections of eggs in which these changes are taking place. A 32-cell stage is shown in Fig. 3; a 54-cell stage (with 6 cells inside) in Fig. 4; a 64-cell stage (with 12 cells inside) in Fig. 5, and a 105-cell stage (with 26 cells inside) in Fig. 6. These eggs were all from the same bunch.

Figs. 3 and 4.



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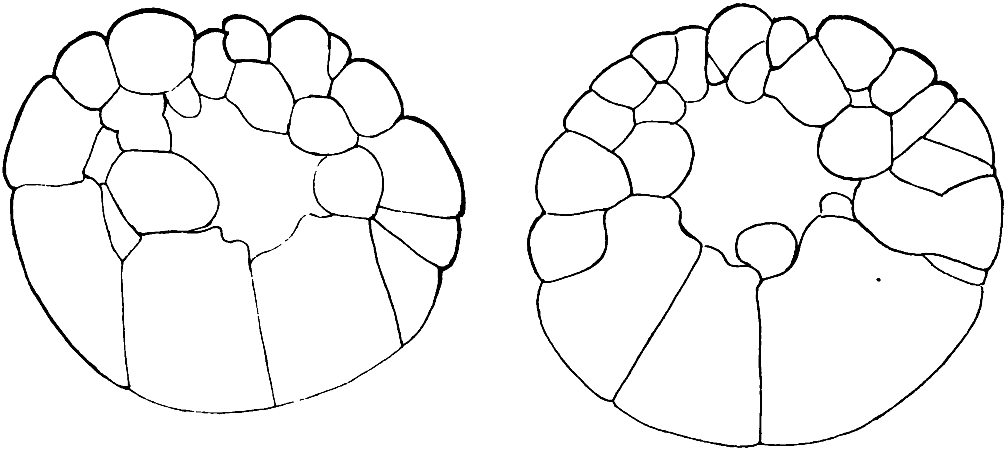
Figs. 5 and 6.



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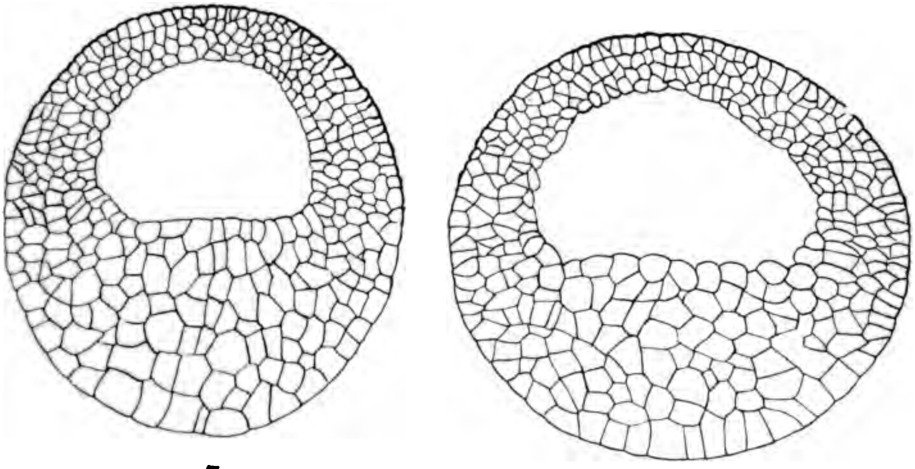
The next series begins with segmentation stages much more advanced than the last. The first two eggs, Figs. 7 and 8, are in the same stage of development. On egg has been flattened, in cutting, in one plane, and the other in the plane at right angles to the former. Unless one is on his guard the different shapes of eggs due to compression in cutting might lead to erroneous conclusions. In fact, the egg itself, at this and at the following stages, is spherical. The roof and sides of the segmentation cavity are rather thick in these two figures. The floor of the segmentation cavity is at about the level of the equator of the egg.

A stage when the blastopore has just appeared is drawn in Fig. 9. The segmentation cavity is still broader from side to side

Figs. 7 and 8.



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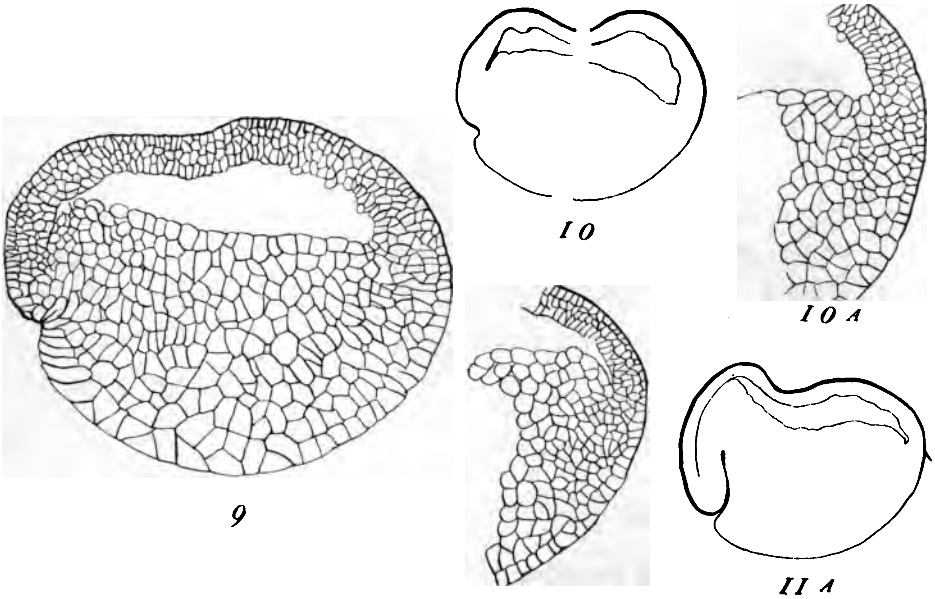
and not so high (the flattening of the egg exaggerating this effect). The yolk mass rises relatively high up on the floor. The roof of the segmentation cavity is thinner. This thinning of the roof can not be entirely accounted for by an increase in area, for, the surface of the egg is not much greater, if at all greater, than in the stage preceeding. We can, however, account for the thinning out on the assumption that the side-walls have been carried further downwards. The relatively higher position of the yolk on the floor of the segmentation cavity must be compensated for in some way, and the postulated downgrowth of the side-walls would make good the space formerly occupied by the yolk. It is, therefore, significant to find at this time a narrow crevice appearing around the sides of the segmentation cavity between the yolk and the ectodermal wall of the segmentation cavity. This crevice first appears at the anterior end, and later along the sides and last at the posterior end. The crevice has been supposed by Brachet and others to represent a split that appears between the yolk and the more superficial cells; but I shall try to show on the contrary that it is due to the pushing upwards of the yolk, rather than to a splitting downwards in the solid wall of the sides of the egg.

The next stage is represented in Fig. 10, in which the gastrulation is a little more advanced. The posterior side of this section is drawn to a larger scale in Fig. 10 *A*. The posterior edge of the roof of the segmentation cavity is seen to be thinner than before, and the smaller and darker cells extend further down on the sides. As yet the crevice has not appeared at the posterior end.

Figs. 9—11.



9

10

10 A

11

11 A

The next stage, Fig. 11 *A*, shows the dorsal lip of the blastopore further advanced. The yolk on this side of the egg is thrown high up into the segmentation cavity, a condition often figured by previous writers, who have not, I think, fully appreciated the importance of this fact[1]). The posterior side of the same egg is shown in Fig. 11.

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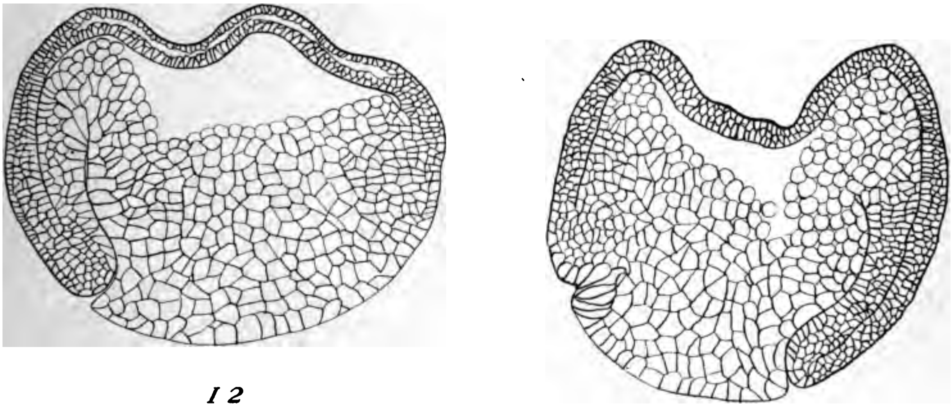
[1]) H. V. Wilson, however, has drawn particular attention to this throwing

Compared with Fig. 10, through the same region, it will be seen that the roof of the segmentation cavity is continuing to thin out. The crevice between the ectoderm and the yolk at the posterior corner of the segmentation cavity has appeared, and in this case, especially, looks as though it were formed by the rolling upwards of the yolk cells.

In the next stage, Fig. 12, the changes noted for the last stage have been carried still further.

Finally in Fig. 13 the posterior lip of the blastopore is well marked (the section is turned in an opposite direction from the last). The crevice at the posterior end between the ectoderm and the yolk is now relatively long; and the yolk at the sides is thrown high up into the segmentation cavity, while the floor of the segmentation cavity has even sunken down[1]).

Figs. 12 and 13.



12

13

In Fig. 14 is represented a late segmentation stage from a different bunch of eggs. The roof of the segmentation cavity is very thin, and the material pressed more outwards and downwards at the sides. A later stage from the same series is drawn in Fig. 15. The segmentation cavity is much broader, and its floor is now pressing somewhat upwards. The side walls of the segmentation cavity are still quite thick, but less so than in the preceeding stages. In this

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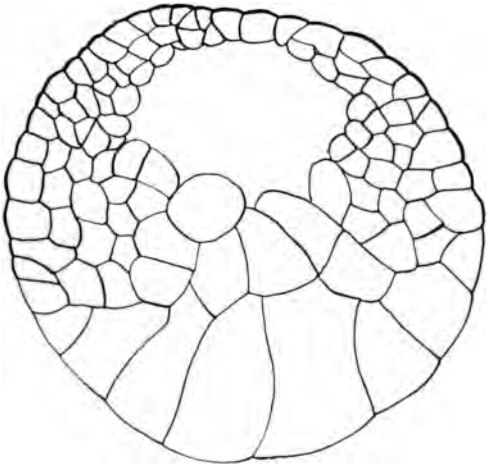
upward of the yolk at the anterior end of the archenteron, and has discussed its meaning.

[1]) The top of this egg has bulged inwards as a result of the action of some of the hardening fluids.

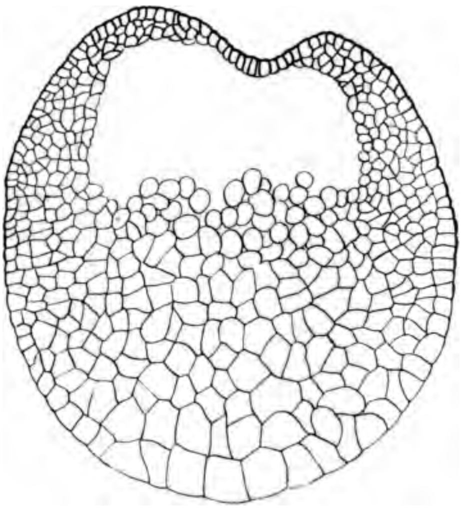
particular bunch of eggs, from which these figures were taken, the contrast between the thickness of the top and sides of the segmentation cavity is greater than usual. In other series the top is relatively thicker. For exact results, therefore, it is important to compare directly only stages from the same batch of eggs, which are much more like each other at every stage than are eggs from different bunches.

In order to study the later stages of gastrulation I have been obliged to resort to another set of eggs. Fig. 16 *A* shows a stage in which the yolk plug is present and much reduced in size. The most striking feature in this section is the position of the yolk mass that has been carried upwards along the inner wall of the segmentation cavity, while the central portion is, it appears, sinking downwards a little. While the yolk plug is being drawn in, the yolk continues to extend upwards around the inner wall of the segmentation cavity, and finally meets near the top. The segmentation cavity becomes reduced in size, and finally sinks more into the interior of the yolk mass, where it finally disappears.

Figs. 14 and 15.



14

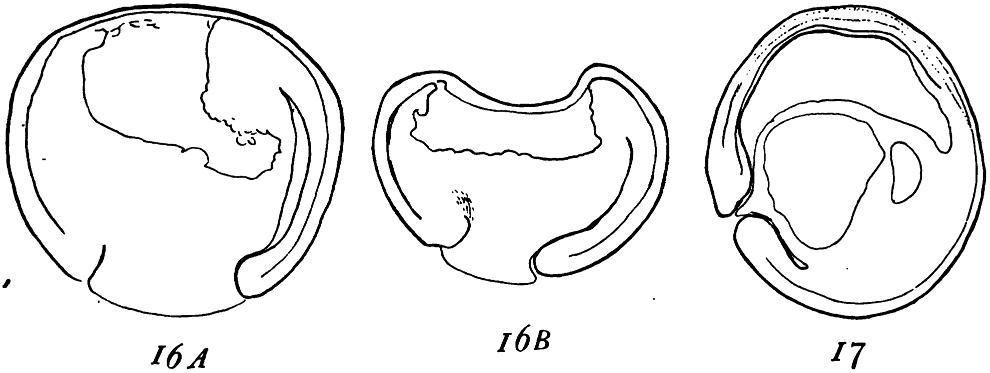


15

A somewhat older stage is represented in Fig. 16 *B*, from another bunch of somewhat smaller eggs. The yolk in front of the archenteron is not carried so high up towards the top of the egg as in the last figure. There is a group of elongated yolk-cells just above the ventral lip of the blastopore that is very conspicuous in the section, and probably indicates an active process of withdrawal of cells in this region.

A stage, when the yolk plug is just disappearing within the blastopore, is shown in longitudinal section in Fig. 17 (turned in the opposite direction from Figs. 16). The archenteron has opened out into a large cavity. The segmentation cavity has become surrounded by yolk, and has sunken into the interior. In fact there are two cavities in the yolk in this egg, both of which appear to be parts of the original segmentation cavity. Such a result might follow from a condition like that shown in Fig. 16 *A*. The ectoderm now covers the whole surface of the egg, and is correspondingly reduced in thickness as a comparison of the figures will show. Much of the ectoderm that formed at first a part of the roof of the segmentation cavity has now been carried far down over the sides of the egg.

Figs. 16 and 17.



16 *A*

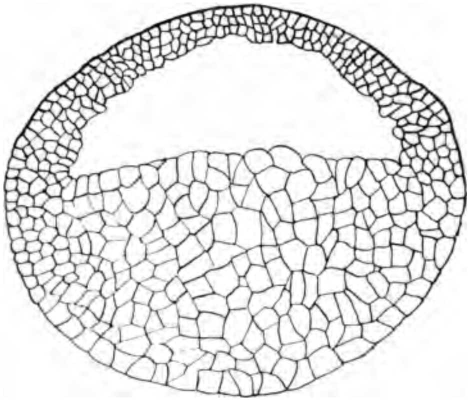
16 *B*

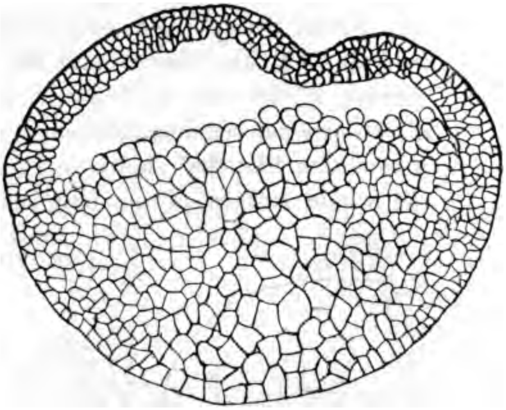
17

All of the preceding sections have been longitudinal ones, i. e., sections through the top of the egg and the middle of the dorsal lip. I shall call cross-sections those that are at right angles to the last, and cut across the middle of the yolk hemisphere. As the lateral lips of the blastopore extend around the egg they will be cut across in these sections. Since the lips appear from before backwards a single series of sections of this sort often shows a series of stages in the process of blastopore-formation with the associated changes in the downward movement of the superficial material.

Fig. 18 is through the middle of an embryo in which the blastopore has just appeared. It is the 45th section from the anterior end in a series of 85. In this region of the egg, the crevice between the ectoderm and the yolk has not yet appeared, although the floor of the archenteron has begun to lift upwards. A little older stage is shown in the Fig. 19 (the 32 section of a series of 70). In this embryo the blastopore had formed a very short crescent. The egg was cut slightly obliquely, so that the right side of the section shows a more advanced condition than the left. The smaller cells at the side appear to have reached a lower level than in the preceding stage.

Figs. 18—20.


18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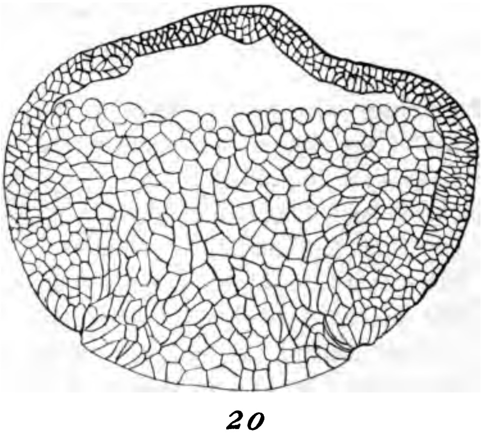
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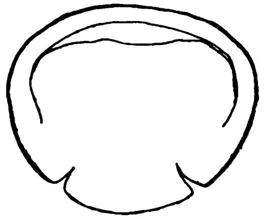
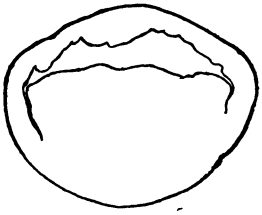
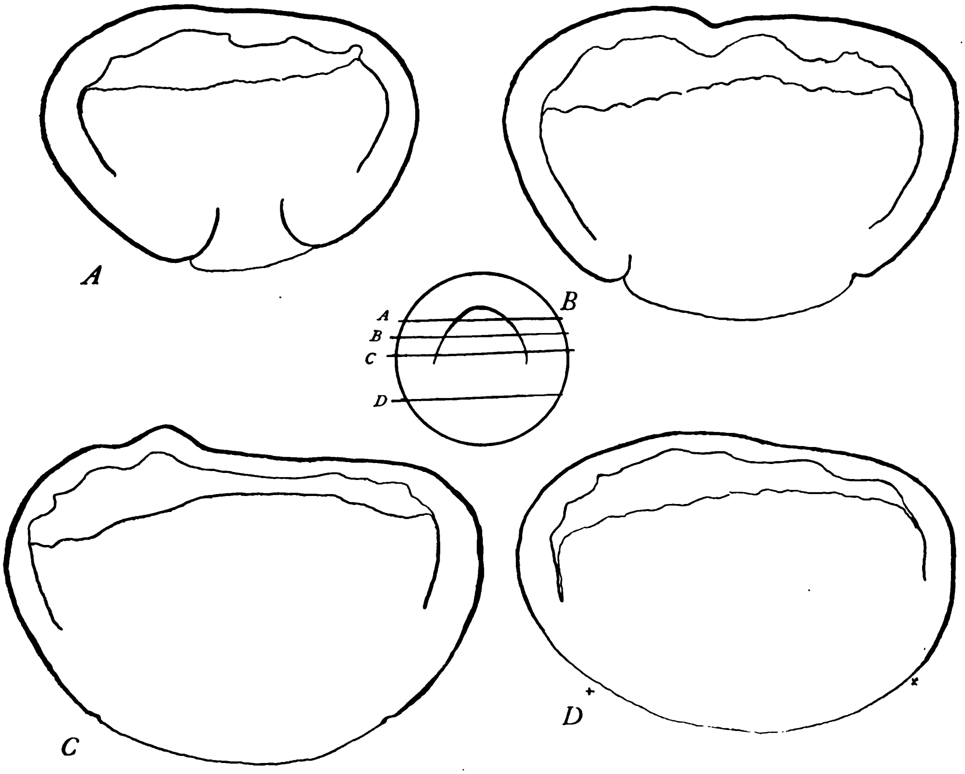

20A


20B

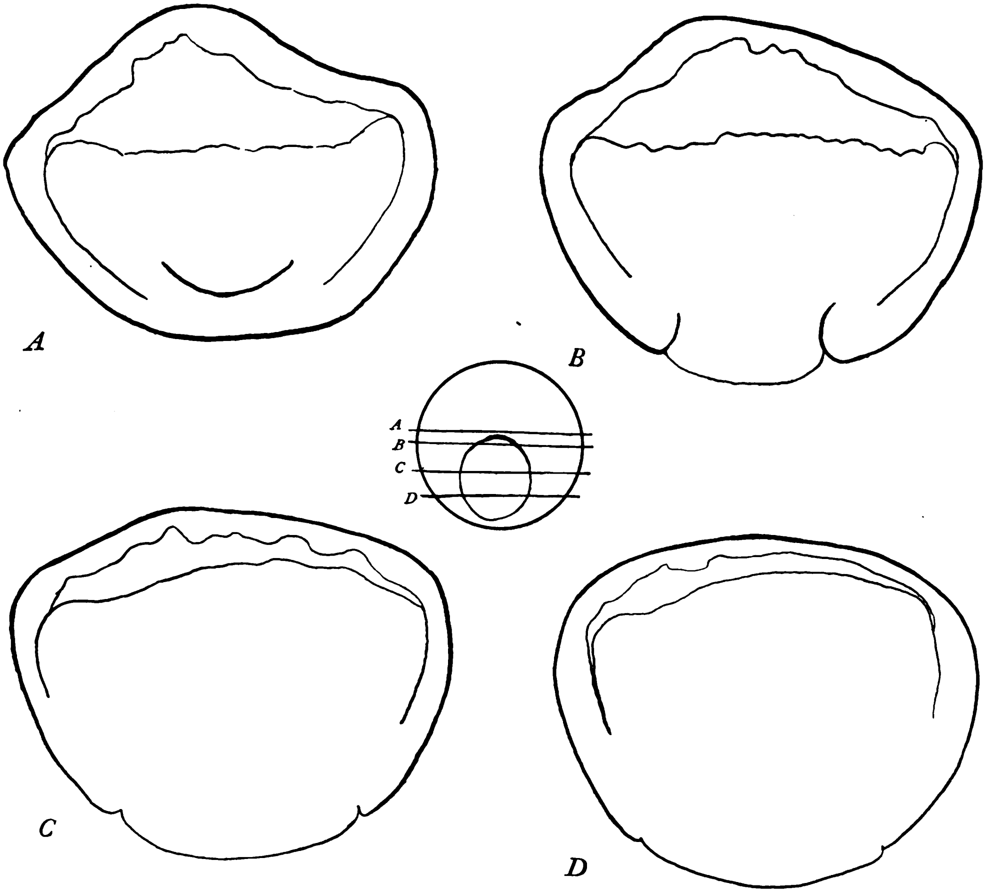
A cross-section of a slightly older embryo is shown in Fig. 20. This is the 20th section (in a series of 54), and, therefore, anterior to the middle of the egg. The inturning at the lateral lips of the blastopore can be seen on each side of the lower part of the section. Comparing this section with the preceding, it will be seen that the small cells at the sides now extend much further down, while the yolk floor rises higher up, and that the crevice between the ectoderm and the yolk is longer. As one passes through this set of sections towards the anterior end, the lateral lips of the blastopore are better developed, and are drawn nearer together, Fig. 20 *A*; the yolk rises higher in the segmentation cavity, and the small cells at the sides appear to cover relatively more of the surface. Passing towards the posterior end the reverse conditions are met with; Fig. 20 *B*. Cross-section through older stages, when the dorsal lip of the blastopore has begun to advance over the yolk, show the following important changes.

Fig. 21.



The four cross-sections shown in Figs. 21 are through an egg in which the blastopore has begun its advance. The first figure *A* is the 5th section posterior to the point where the lateral lips fuse in the dorsal lips. The lateral lips are here near together, and the crevice between the ectoderm and the yolk is long. The next section *B* is the 14th from the blastopore, and shows the lateral lips much further apart. The third section is the 35th of the series, and, near the middle of the egg. It will be observed how much further out the lips of the blastopore are at this level. The last section is the 60th, and in it the lateral lips are not developed. Their positions can be approximately located by means of the mesoderm, and are indicated by the two crosses. These points lie relatively still further out at the sides. Thus as the lateral lips advance to become in-

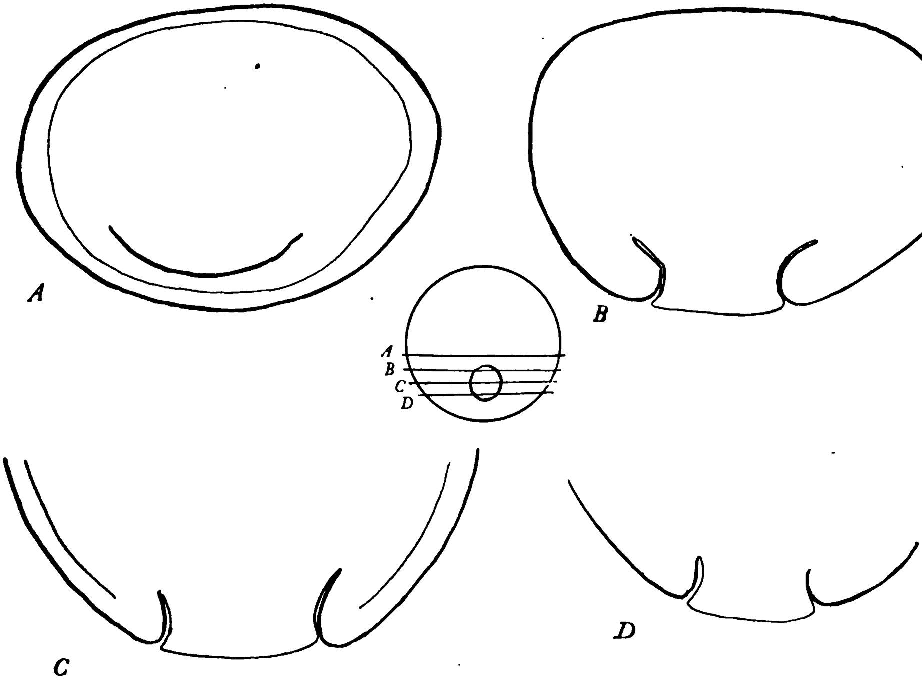
Fig. 22.



corporated into the dorsal lip, the material advances toward the middle line. A continuous advance of all the material at the sides must take place, including the invaginating region itself.

The next series, Fig. 22, is through a later stage. The first section, *A*, is the second in front of the one where the dorsal lip opens out at the surface. The archenteron appears as a narrow slit below the surface (on the lower side of the figure). The second section is the fifth one posterior to the dorsal lip, and the 28th of the series. The lateral lips have approached. If we imagine them as meeting in this section, and the yolk plug withdrawn, the width of the archenteron, as shown by section *A*, would now correspond to the distance between the outer ends of the archenteric invagination at the sides. The third figure, *C*, is the 37th of the series. The lateral lips are much further apart and less developed. The distance between them is much greater than the future width of the archenteron at this level. The fourth section, *D*, is the 45th of the series, and much like the last. It lies at the level where the lateral lips

Fig. 23.



begin to round in towards the posterior end. There are 20 more sections in the series.

The last series Fig. 23 is through an egg in which the blastopore is nearly circular, and much reduced in size. The first section, *A*, is in front of the external opening of the dorsal lip, and shows the archenteron. The next section, *B*, is the second behind the opening of the dorsal lip, and shows the lateral lips near together. The third section, *C*, is through the middle of the blastopore, and shows that here too the lateral lips are now near together, and that compared with Figs. 20 and 21, the approach is greater than can be accounted for by the depth of the archenteron at the sides, so that the whole region must be imagined to have pushed downwards. The fourth section, *D*, is through the posterior end, where the lateral lips are even nearer together than in the preceding.

If these three sets of sections are compared with the earlier stages shown in Figs. 18, 19, it will be seen, that the lateral invaginations, that appear along the lateral lips are much further out at the sides when they first appear than in the later stages, and that the surface between them is greater than the width of the archenteron when it first appears. There must be postulated, therefore, an advance of the sides, including the lateral lips and their invagination, during the closure of the blastopore. This movement appears to be only a continuation of that which has been taking place during the earlier stages.

Provisional Interpretation of the Early Stages.

In the preceding account I have confined myself largely to a description of the changes that take place during successive stages, only intimating at times the character of the processes that bring about the changes. I shall now attempt to give a continuous account of the way in which I suppose the downgrowth of the material to take place. It will be convenient to classify the process under three headings, although in the actual development the process is a continuous one.

First period: The transportation of the material during the cleavage process.

Second period: The changes just prior to and during the appearance of the blastoporic rim.

Third period: The drawing together of the lips of the blastopore.

First Period.

The egg increases a good deal during the segmentation period as would be expected since the large segmentation cavity develops at this time. If the fluid in this space was merely squeezed out of the blastomeres, the egg might remain the same size, although a cavity formed in its interior. That something is contributed to the segmentation cavity by the surrounding blastomeres is probable, since the fluid coagulates in the hardening reagents, although it is probable that the greater part of the fluid is due to absorbtion of water from the exterior; but whether this water first enters the blastomeres, and is secreted by them into the interior, or whether it enters more directly is quite unknown.

The third meridional cleavage separates largely the material that becomes the roof of the segmentation cavity from that of the floor. It is also probable that some of the material that forms the side walls of the segmentation cavity may come from the upper ends of the four lower cells of the eight-cell stage. From the 32-cell stage onwards, some of the cells of the roof of the segmentation cavity pull into the interior, and apply themselves to the inner wall, especially to the sides. As the segmentation procedes the material of the roof spreads out, and is carried downwards towards the equator of the egg, so that some of the material that originated higher up on the sides, and, especially, the cells which drew into the interior, become carried further downwards. As this change goes on throughout all the later cleavage periods, the lateral material is carried to, or even below the equator of the egg. At this time the segmentation cavity changes its shape, spreading out more laterally, as the floor rises up higher. As a result of the latter change, room is made for the slipping downwards of cells of the walls of the segmentation cavity. The roof also becomes gradually thinner. In part this thinning out is due to the somewhat increased area of the roof, but also to the extension downwards of the sides.

There is another possible interpretation of the changes that take place during this period, which is the one usually adopted by most writers who have paid any attention to this period of the development. It is usually stated that the superficial material of the egg in the region of the equator continues to divide, and that the smaller cells formed in this way add themselves, as it were, to those of the top, so that the latter appear to extent further downwards. It is stated, also, that the crevice that appears between the ectoderm and the yolk is due to a split that separates the outer from the inner cells. I have examined these possibilities with some care, and the evidence seems to me to point unmistakeable to the conclusion that the crevice is not a split, but is formed as a result of the ectoderm slipping down as the yolk pushes upwards. Thus one hypothesis will account for the shifting downwards of the material of the surface, for the formation of a crevice where the two regions are moving in opposite directions, and for the rising up of the floor of the segmentation cavity.

The darkening of the egg that takes place during the later cleavage period, which I called attention to in 1891, is due to the movement of darker cells downward, and is not due, primarily, as I supposed, to the development of the pigment in the cells around and below the equator of the egg.

Second Period.

The preceding account has anticipated to some extent the changes that take place during this second period. The changes begin when the yolk floor rises up in the segmentation cavity, causing the appearance of the crevice around the sides. The period culminates with the appearance of the blastoporic rim. The changes begin at the anterior end, where the dorsal lip of the blastopore appears, and slowly extend around the egg. Just after the appearance of the rim, the movement downwards seems to be more rapid, although I can not bring forward a conclusive proof that this is the case. The change in shape of the yolk cells near the region at which the rim is to appear is significant.

Third Period.

As soon as the blastoporic rim appears the subsequent transfer downwards of the material can not be mistaken. The edges of the lip advance, rolling in somewhat as they advance, so that a few of the cells situated just outside and on the lip may be seen to roll under. At the same time the whole yolk field begins to move upwards under the advancing lips.

The dorsal lip appears first and advances rapidly backwards, while the lateral lips are forming. In fact, the advance of the dorsal lip is largely due to the incorporation into it of the material that is being constantly brought up to the middle line by the lateral lips.

As the lateral lips develop faster nearer the anterior end, the result is that the dorsal lip appears to advance more rapidly backwards than the lateral lips advance from the sides, but there can be little doubt that this backward advance is not due, to any extent, to the material that first appears at the dorsal lip moving backwards, but to the lateral lips coming up to the middle line. When the blastopore has been reduced to a small circular or elongated opening the advance is more nearly equal from all sides. The ventral lip undergoes an exactly similar series of changes, so that the yolk surface is not only slipping forward under the dorsal lip, but under the sides and under the posterior lip also, as KOPSCH's photographs have shown.

Measurements of the egg at the time when the dorsal lip first appears, and after the blastopore has just closed, show that the egg does not increase in size during this period. This fact has an important bearing on the interpretation of the changes that are taking place. It shows, in the first place, that the thick blastoporic rim does not rise up over the lower surface of the egg, but that the latter slips in under the blastoporic rim. Equally important is the bearing of this fact on the length of the embryo that is formed. If the point at which the dorsal lip appears on the surface be indicated, and the point at which the posterior lip first appears be also marked, it will be found that the region covered by the blastopore is somewhat longer than the length of the dorsal side of the embryo. If to this length we add that of the head region of the embryo, which experiments indicate develops in front of the first position of the dorsal lip, then the embryo-forming material appears to extend over a much longer region of the egg than that actually covered by the embryo. This condition has lead to endless confusion. The explanation is found in the fact, that, as the yolk draws into the interior of the egg the surface material out of which the embryo is to develop is actually as a whole drawing somewhat together, i. e., the downward movement involves a real drawing together of the material at both the anterior and posterior ends as well as at the sides. This point will be clearer when some other changes in the development have been considered.

During the period of overgrowth the ectoderm gradually becomes thinner, owing to the greater area it has to cover. The mesoderm is carried towards the middle line and forms a thin sheet over the dorsal surface. In the dorsal lip itself there is present at all stages a relatively large amount of material, not because the cells there are multiplying and growing more rapidly then elsewhere, but because the material from the sides is being continually brought up to the dorsal lip.

Kopsch has given some diagrams to illustrate his view of the location of the material that goes to form the embryo, and to show how this material is carried into the embryo. He also tries to show that Roux's theory, namely, that the second plane of cleavage usually lies approximately between the material that goes to form the anterior and the posterior halves of the embryo, is erroneous or impossible. Kopsch's diagrams illustrate very well, it seems to me, the location of the material at the time when the posterior lip of the blastopore is about to develop, and the later stages that follow, but in this respect the diagrams give little that is new. They fail to show, however, the earlier location of the material, and for this reason fail to show that Roux's theory is incorrect. On the contrary, I think, Roux's view gives a much more approximate idea of the relation of the embryo-forming substance to the position of the second plane of cleavage than does that of Kopsch.

The Origin of Ectoderm, Mesoderm, and Endoderm.

The primary object of this paper is not to consider the question of the origin of the germ-layers of the embryo, but to trace the movements of the materials. Since, however, the two questions are closely related, a short account may be given of the origin of the layers.

Ectoderm. The roof and the walls of the segmentation cavity become the outer covering of the embryo. The transfer downwards of this material has already been sufficiently described. The surface cells, that lie just below the walls of the segmentation cavity, also form part of the ectoderm, but, as I have tried to show, most of these cells have been relatively higher up on the egg during the early segmentation and blastula stages. Experiments with the eggs indicate that the ectoderm arises from the upper four cells of the eight-cell stage and also from the upper part of the lower four cells, but the exact limits with reference to the third plane of cleavage is difficult to determine.

Mesoderm. The cells of the mesoderm can first be recognized as such at the time of the appearance of the dorsal lip of the blastopore. As the lateral lips develop the mesoderm also appears around the egg in the same relative position in regard to the blastoporic rim that it occupies in the dorsal lip. When first recognizable the mesoderm is made up a group of cells just below the »crevice«, and between the ectoderm and the yolk. It is from the beginning directly continuous with the inner layers of the wall of the segmentation cavity, and often presents the appearance of developing directly out of these cells as they push downwards. Even after the blastopore lips have formed and are closing in, there is always a close connection between the inner layers of the ectoderm and the mesoderm at the edge of the rim as though cells from the former were pushing in to become mesoderm as the lateral lips become incorporated in the dorsal lip. On the other hand a part of the group of mesoderm cells resembles so closely the yolk cells in the region in which the mesoderm first appears, that it seems possible by a single division taking place to convert the yolk cells into mesodermal cells. It is not improbable that some of the mesoderm arises also in this way. The three possible sources that I have given are not mutually exclusive, for, the cells of the inner wall of the segmentation cavity are directly continuous with the yolk cells in the region where the mesoderm is formed, so that the distinction between them may be an arbitrary one.

The group of mesodermal cells is carried downwards in the dorsal lip of the blastopore, or rather, the lateral lips bring up their mesoderm to the mid-dorsal line to add it to the mesoderm already there. In this way the mesoderm comes to lie over the dorsal surface of the embryo, beneath the medullary plate.

I have already referred to the tongue of cells that grows upwards around the inside of the dome-shaped roof of the segmentation cavity. This is the material out of which the mesoderm of the sides, and ventral surface of the embryo is subsequently formed. The material arises in close proximity to the cells that produce the first formed mesoderm, although the divisions that transforms them into mesoderm do not occur until a relatively late stage.

The »tongue« of cells appears to be formed by the yolk cells pulling upwards at and near the region of the top of the archenteric invagination, which takes place entirely around the egg. The part of the mesoderm formed in this way represents, therefore, inwandering endoderm cells.

Endoderm. The yolk makes up, of course, the main bulk of the endoderm. The point of chief interest at present is the origin of the cells of the roof of the archenteron. This is a difficult question, for a discussion of which we still lack, perhaps, sufficient data, although some points, at least, can be made out with a high degree of probability.

The origin of the slit-like archenteron first demands attention. What can actually be observed may be stated in a few words. A depression, or pit, appears in the yolk cells just below the region where the mesoderm can be identified; Fig. 9. In surface view the pit is deeply pigmented, and sections show that this is due to the pigment that accumulates in the outer, pointed ends of the cells, that appear, from their shape, to be drawing in from the surface.

There is present in sections a line of superficial cells just above the pit that more nearly resemble the yolk cells than the ectoderm higher up, although they grade off insensibly into the latter. These cells roll around into the pit, as the lip of the blastopore develops, and form, therefore, a part of the dorsal wall of the archenteron. This same process occurs not only at the dorsal lip, but around the whole rim of the blastopore. When the lateral lips are brought to the middle line these cells, that have rolled under its edge, will form the cells of the mid-dorsal wall of the archenteron.

As the blastopore extends downwards the pit becomes a narrow slit, Fig. 12. At the top of the slit there can always be found a group of wedge shaped cells with their pointed ends turned outwards and downwards. These cells are exactly like those that first appeared when the dorsal lip developed. A crucial point is whether these cells are the same ones that first appeared, or whether new ones have continually assumed the shape and the position of those that preceded them. There is, in fact, some indirect evidence from the form of the different cells at the top of the archenteron to lead one to the conclusion that some of the pointed cells become added to the dorsal wall of the archenteron, while others that lie on the anterior end of the floor become successively pointed, move to the anterior end, and in turn may be added to the dorsal wall. Most of them, however, in all probability move further upwards into the yolk. The determination of just what changes are taking place at the top of the archenteron is extremely difficult, but the inturning of the yolk during the period of gastrulation, combined with the observed shape of the yolk cells at the top of the archenteron, make it probable, that some of the superficial yolk cells that disappear under the advancing rim of the blastopore must become incorporated in the yolk mass at or near the top (and at the sides of the lateral rim, and at the top of the posterior rim) of the archenteron. The actual number of cells across the lower hemisphere between the points where the lateral lips appear is actually greater than the number forming the cross-section of the floor of the archenteron. The rolling around of cells at the anterior end of the archenteron makes the archenteron longer in this direction than the actual space overgrown by the dorsal lip, so that the ultimate number of cells forming the floor in this direction may be greater than the number over the corresponding surface area of the yolk field.

There is another point in favour of the view that the cells of the floor of the archenteron change shape at the anterior end, one after another. In this way we can account for the movement of the yolk surface under the lips of the blastopore. As each cell changes its shape in this region, so that its end next to the archenteron becomes smaller, it occupies less room, and the line of cells lower down are drawn further up towards the upper end. As each approaches the turning point, it also changes shape, and exerts a similar drag on the cells lower down. I suggest this view with some hesitation, for it seems undesirable to add another account to the many that are already in existence to explain the process of gastrulation of the frog's egg; but as a working hypothesis, and one that is accord with the most recent results I trust it may at least be given a serious hearing.

To sum up: The anterior cavity of the archenteron is lined both above and below by the yolk cells. This part of the archenteron has been formed by the drawing upward of the yolk cells some of which roll around the anterior end of the archenteron. The mid-roof of the rest of the archenteron is formed by the cells that have rolled under the rounded lips of the blastopore, and have been brought to the middle line by the advance and fusion there of the lateral lips of the blastopore. The side walls of the archenteron, except for its anterior end, are formed by the yolk cells that draw in along the lateral lips of the blastopore, and also possibly to a limited extent by cells that have moved around the upper end of the archenteric cavity of the lateral lips. The side walls, as a whole, also are brought nearer to the middle line by the advance of the lateral lips.

The »Mechanics« of the Downward Movement of Material.

The postulation of an extensive movement of material, such as that which I have attempted to trace, invites, almost demands in fact, an attempt to account for the mechanics of the process. I shall attempt, therefore, to give a provisional account of how the process takes place, admitting freely that the difficulties involved in an attempt of this sort with so little data at hand make the hypothesis far from satisfactory.

The first beginning of the downward movement of the material at the top of the egg is associated with the decrease in thickness of the walls, and with the enlargement of the segmentation cavity. That the enlargement of the cavity itself is the mechanical means by which this change is affected seems to me improbable. On the contrary its enlargement appears to be the result of the changes in its roof and floor, and not the cause of them. The results must, therefore, be due to the activity of some region of the egg-substance. The thinning out of the roof suggests that the pressure of its small cells may cause its sides to push downwards against the less resisting yolk. On the other hand, if it were simply a question of increased pressure in the roof it is not clear why the roof might not simply turn into the segmentation cavity, and thus relieve the pressure. If, however, the yolk cells responded actively to the increase in pressure from above, and changed their shape, a re-adjustment might be continually taking place in this way. In fact, the movement upwards of the whole floor of the archenteron suggests that a re-adjustment of some sort is going on. The assumption would be simpler, however, if we postulate only a change of shape in the yolk cells, which pushing upwards allow the sides of the egg to slip downwards. This downpull causes all of the cells of the roof of the segmentation cavity continually to readjust themselves to the new conditions, and the roof becomes thinner. The re-adjustment may not be simply a passive process, but the cells may each respond to the local conditions of tension of the region in which they lie. Whether, then, both the yolk cells and the surface cells of the roof are actively engaged in the downgrowth, or the yolk alone is responsible, while the cells of the roof only actively readjust themselves to the conditions, can not be stated. By removing the roof of the segmentation cavity in the living egg this point might be determined.

At the time of appearance of the blastopore there can be little doubt that a change in shape of the yolk cells is largely responsible for the changes that take place, and if my account of the inturning of the yolk into the blastopore is correct, this change of shape of the cells may entirely account for the results. It may be that the cells of the blastoporic rim also take an active part in the process of overgrowth of the lower hemisphere, by changing shape and do not simply draw together after the fashion of an elastic band as the yolk withdraws. In fact, there must be very extensive changes in the relative positions of the cells to each other during this time, and these changes also can be best accounted for on the assumption of the contractile power of each cell in response to the surrounding conditions of tension or of pressure of the parts. The process may not be dissimilar in kind from that by which artificially exposed surfaces of later embryos are covered over. I have tried to show that this process also must be due to a process of contraction of the cells.

That the rubber-band idea of the contraction process is insufficient is also made probable by a number of other considerations. The drawing together of the blastoporic rim begins at the anterior end, and goes on there for some time before the rest of the rim is formed. Moreover the movement is for some time towards the anterior end, as well as towards the middle line, so that the movement goes on in a definite direction, and is not equal in all directions. If we consider the process of contraction as due to a response of the individual cells we get quite a different conception of the process than when we compare it to the process as seen in a stretched band. In fact the phenomenon is an internal one, and the conditions are not impressed on the regions from without. If the molecules of a piece of rubber could contract or expand locally the process would be somewhat more like the results caused by a change in shape of the cells. The power of the individual cells to change shape by a process of contraction, comparable to that of a muscle, gives the peculiar character to many of the phenomena of development. Whether the phenomenon of contractility which appears to be one of the fundamental characteristics of living things is in its ultimate analysis a »vital« or a mechanical process does not concern us here, but I do not think it probable that the change in shape of the cells that takes place during development can be explained, as **Rhumbler** has tried to show[1]), as due to differences in the osmotic and other physical conditions on different sides of the same cell, but rather to a process of contraction like that shown by a muscle cell, whatever the ultimate character of the process may be.

It would be tempting to extend this view to many of the developmental phenomena, for, an examination will show that a large number of the changes through which the embryo passes may be accounted for on the assumption that the individual cells have the power to change their shape by certain parts of the cell contracting in response to local conditions, internal and external. I need scarcely do more then name the different forms under which this phenomena appears to occur: The cleavage of the egg is probably a contractile process; the inwandering and immigration of amoeboid cells belong to this category, if we may extend to this process the same explanation that Jennings has recently discovered for amoeba. The invagination and the formation of pouches of many kinds can also be explained as the result of the change in shape of the cells of a given region. The rounding up of solid organs and the delimitation of groups of cells, in general, may be accounted for in the same way. In fact, may we not ask ourselves whether the so called formative forces, or factors, or principles (whichever term we prefer) may not be an expression of the phenomenon of response and contraction?

Summary.

1) The frequent appearance of abnormal embryos in the upper hemisphere of the egg can be explained either on the grounds that the abnormal and the normal embryo are developed out of different material, or else that the embryo-forming material lies at first higher up in the upper hemisphere, than has heretofore been suspected, and that it is carried downwards below the equator of the egg before the blastopore appears. These alternatives could be tested either by removing two or four of the upper blastomeres in the eight-cell stage (the results have been described in the VI-paper of this series), or by a reexamination of the early stages of normal development. The results of such an examination are given in the present paper.

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[1]) I wish to state however that I owe much to **Rhumbler**'s clear analysis of the nature of the factors involved in the gastrulation process.

2) The segmentation cavity grows larger during the early cleavage period and during this time its roof is becoming thinner, Figs. 2—8. Towards the close of this period, the floor of the segmentation cavity rises higher up, and the cavity becomes in consequence broader horizontally, Figs. 9—10. At the same time that the yolk mass moves upwards, the lateral walls of the segmentation cavity push downwards over the sides of the egg. The crevice that appears in the interior between the yolk and the ectoderm, Figs. 9—13, appears when the processes just described have reached a certain stage. The crevice owes its origin to the rolling upwards of the yolk mass as the lateral walls push downwards.

3) The appearance of the blastopore is due to certain yolk cells changing shape, which causes at first an indentation of the surface and leads later to the formation of the slit like archenteron in the yolk. This process continuing at the anterior end, an extension forward of the archenteron into the yolk is brought about; the yolk mass during the same time pushes upwards higher into the segmentation cavity, Fig. 12. The shape of the cells at the top of the blastoporic invagination suggests that in a change of shape may lie the cause of the moving of the surface cells of the lower hemisphere under the advancing lips of the blastopore. As each cells reaches the top of the invagination it becomes pointed at its outer end, and as the cell changes shape the next cell is drawn higher up in the interior. The new cell then undergoes a similar change in shape, and the process repeats itself until all the surface cells have been drawn under the lips of the blastopore. The interior yolk cells of the lower hemisphere may also draw upwards by changing their shape.

4) The ectoderm of the embryo is formed from the cells of the roof and sides of the segmentation cavity that are pushing downwards during the entire cleavage period, and are later carried over the lower hemisphere by the advance of the blastopore lips. The mesoderm also arises high up in the egg. It arises in part from cells that in the early cleavage formed part of the inner wall of the roof of the segmentation cavity. From their beginning these cells are continuous with the yolk cells at the lower edge of the segmentation cavity, and these cells also appear to be added to the mesoderm. Thirdly, the inner layer of the ectoderm at the edge of the blastopore rim is always continuous with the mesoderm, and as the blastopore closes it is not improbable that some of the inner ectoderm cells are added to the mesoderm.

The anterior portion of the walls of the archenteron is lined by yolk cells, and is formed by the rolling around of yolk cells at the top of the archenteron. The floor of the remaining portion is the yolk of the lower hemisphere. The roof of the mid-dorsal part is formed by cells that have rolled around the edge of the blastopore rim and have been brought, for the most part, to the middle line by the advance of the lateral lips. The lateral walls of the archenteron are lined by the cells that first draw in at the rim, especially at the lateral lips; possible also some of these cells come from cells that by changing shape at the top of the invagination have come to pass around the upper end of the archenteric split to be added to the outer walls.

5) The »mechanics« of the downward movement of material finds its explanation in the power of the yolk cells to change shape. The entire movement of the floor of the segmentation cavity can be explained in this way. The change is especially noticeable at the blastoporic invagination. The downward, compensatory movement of the outer ectoderm cells may be due to a passive re-arrangement, or the cells may also take an active part in the process. Which of these processes occurs can not be stated.

The power of individual cells to change their shape is due to their contractile power which is no doubt the result of a stimulus, but the real nature of the phenomenon of contractility is not known at present, although it does not appear to correspond to any one, known physical process. It is suggested as a tentative view that the so-called formative principle may be also a expression of this contractile power.

Zusammenfassung.

1) Das häufige Erscheinen unnormaler Embryonen auf der oberen Eihemisphäre kann entweder auf der Grundlage erklärt werden, daß normaler und unnormaler Embryo sich aus verschiedenem Material entwickeln, oder aber daraus, daß das embryobildende Material weiter nach oben an der oberen Hemisphäre gelegen ist, als man bisher annahm, und daß es bis unter den Eiäquator vor dem Auftreten des Blastoporus hinabgelangt. Diese Alternative läßt sich erhärten: entweder durch Entfernung von zwei oder vier der oberen Blastomeren im Achtzellenstadium (die bezüglichen Ergebnisse sind in der VI. Arbeit dieser Serie niedergelegt), oder durch ein Wiederdurchprüfen der frühen normalen Entwicklungsstadien. Die Ergebnisse einer solchen Nachprüfung sind in gegenwärtiger Arbeit niedergelegt.

2) Die Furchungshöhle vergrößert sich während der frühen Furchungsperiode, indem sich währenddessen ihr Dach mehr und mehr verdünnt, Fig. 2—8. Gegen Ende dieser Periode erhebt sich der Boden der Furchungshöhle und damit gewinnt die letztere eine größere Horizontalausdehnung, Fig. 9—10. Zur selben Zeit, in der sich die Dottermasse aufwärts bewegt, dringen die lateralen Furchungshöhlenwände nach unten über die Eiseiten vor. Der Spalt, der sich im Innern zwischen dem Dotter und dem Ectoderm zeigt, Fig. 9—13, tritt auf, wenn die eben beschriebenen Prozesse ein gewisses Stadium erreicht haben. Der Spalt leitet seine Entstehung vom Aufwärtsrollen der Dottermasse her, während die Seitenwände nach unten vordringen.

3) Das Auftreten des Blastoporus ist durch die Gestaltveränderung bestimmter Dotterzellen veranlaßt, welche zuerst eine zahnförmige Kerbe der Oberfläche verursacht und später zur Bildung des schlitzförmigen Archenteron im Dotter führt. Durch das Weitergehen dieses Prozesses am Vorderende wird eine Ausdehnung des Archenteron im Dotter nach vorn bewirkt; während derselben Zeit drängt die Dottermasse nach oben höher in die Furchungshöhle hinauf, Fig. 12. Die Gestalt der Zellen am Scheitel der Blastoporuseinstülpung legt nahe, daß in dieser Gestaltveränderung die Ursache der Bewegung der der unteren Hemisphäre angehörigen Oberflächenzellen unter die vorrückenden Blastoporuslippen liegt. Beim Erreichen des Einstülpungsscheitels spitzt sich jede Zelle am äußeren Ende zu, und mit der Gestaltveränderung der Zelle rückt die nächste im Innern weiter nach oben. Die neue Zelle unterliegt dann einer ähnlichen Gestaltveränderung und der Prozeß wiederholt sich, bis alle Oberflächenzellen unter die Blastoporuslippen gezogen worden sind. Die inneren Zellen der unteren Hemisphäre können gleichfalls unter Gestaltveränderung nach oben rücken.

4) Das Ectoderm des Embryo bildet sich von den Zellen des Furchungshöhlendaches, welche während der ganzen späteren Furchungsperiode nach unten vorrücken und später durch das Vorrücken der Blastoporuslippen über die untere Hemisphäre transportiert werden. Auch das Mesoderm entsteht weiter oben im Ei. Es entspringt teilweise von Zellen, die in der frühen Furchungsperiode der inneren Wand des Furchungshöhlendaches angehörten. Von ihrer Entstehung an hängen diese Zellen mit den Dotterzellen am unteren Rande der Furchungshöhle zusammen und diese Zellen scheinen gleichfalls zum Mesoderm beizutragen. Drittens ist stets die innere Ectodermlage am Rande der Blastoporusspalte im kontinuierlichen Zusammenhange mit dem Mesoderm, und wahrscheinlich treten beim Blastoporusschluß einige von den inneren Ectodermzellen dem Mesoderm bei.

Der vordere Teil der Archenteronwandung wird von Dotterzellen begrenzt und entsteht durch das Eintreiben von Dotterzellen am Archenteronscheitel. Der Boden des übrigen Teils ist der Dotter der unteren Hemisphäre. Das Dach dieses Teils wird von Zellen gebildet, welche um den Rand des Blastoporusspalts herumgerollt und zum größten Teil durch das Vorrücken der seitlichen Lippen in die Mittellinie gebracht wurden. Die seitlichen Archenteronwände sind von den Zellen begrenzt, die zuerst im Spalt, speziell an den seitlichen Lippen, eingezogen wurden. Wahrscheinlich stammen einige dieser Zellen auch von Zellen ab, die unter Gestaltveränderung am Einstülpungsscheitel um das obere Ende des Archenteron herumgelangt, sich trennen und zu den Außenwänden hinzutreten.

5) Die »Mechanik« der Materialabwärtsbewegung findet ihre Erklärung in der Fähigkeit der Dotterzellen zur Gestaltänderung. Die ganze Bewegung des

Furchungshöhlenbodens läßt sich so erklären. Die Veränderung ist speziell bemerkenswert an der Blastoporuseinstülpung. Die kompensatorische Abwärtsbewegung der Außenzellen kann durch ein passives Wiederordnen ihrerseits veranlaßt sein, oder sie können an dem Prozeß aktiv beteiligt sein. Welcher von diesen Prozessen eintritt, läßt sich nicht feststellen.

Die Fähigkeit der Zellindividuen zur Gestaltveränderung beruht auf ihrer Kontraktilität, die ohne Zweifel einem Reiz gehorcht, aber die wahre Natur des Kontraktilitätsphänomens ist zurzeit nicht bekannt. Doch hängt sie anscheinend mit keinem bekannten physikalischen Prozeß zusammen. Als versuchsweise Auffassung sei angedeutet, daß das sogenannte gestaltbildende Prinzip gleichfalls eine Form dieser Kontraktionsfähigkeit sein könnte.

[Reprinted from BIOLOGICAL BULLETIN, Vol. IX., No. 2, July, 1905.]

THE FORMATION OF THE FIRST POLAR SPINDLE IN THE EGG OF BUFO LENTIGINOSUS.

HELEN DEAN KING.

In a previous paper on "The Maturation and Fertilization of the Egg of *Bufo lentiginosus*" (King, 10), the formation of the first polar spindle and the subsequent divisions of the chromosomes were very incompletely described owing to a lack of material showing the details of these processes. During the spring of 1899, a large number of toads were collected soon after they had emerged from their hibernation, and from three of them sufficient material was obtained to give a more complete history of the late maturation processes. A short account of my study of this period in the development of the egg has already appeared (11); a detailed account is given in the present paper.

I. MATERIAL AND METHODS.

As soon as possible after the toads were captured they were killed by pithing and the body opened at once to ascertain the condition of the ovaries. In a great majority of cases the eggs were found free in the cœlomic cavity and were, therefore, of no use for the purpose intended, as previous investigations had shown that eggs which have broken through the wall of the ovary invariably contain a fully formed maturation spindle lying at the periphery near the center of the black hemisphere.

In several instances, all of the eggs were still attached to the walls of the ovaries when the toad was killed. In these cases some of the eggs were put at once into a dish of fresh spring water and the rest were left in the body of the female which was kept in a moist chamber. A few eggs from each of these series were then fixed at intervals of ten minutes for a period of several hours. By opening an egg under a dissecting lens after it has been taken from the fixing solution and put into 50 per cent. alcohol, one can tell definitely whether the late maturation processes have begun or not; for, if the nuclear membrane in still intact, the nucleus retains its rounded form and can be readily separated from the rest of the egg contents. If one hour after the toad is killed, an examination of freshly fixed eggs shows that the nucleus is still intact, the entire set of eggs can be discarded, as it has been found that further development does not take place unless the germinal vesicle breaks down previous to this time, although the eggs, whether kept in water or in the body of the female, show no signs of disintegration for many hours.

In one case the germinal vesicle was just breaking down when the eggs were first examined under the dissecting lens; in another set of eggs the germinal vesicle could no longer be dissected out half an hour after the toad was killed. These two lots of eggs gave overlapping series of stages which corresponded in every respect. A third set of eggs showed no signs of the germinal vesicle when first examined, and when sectioned showed maturation processes identical with those taking place in eggs which had been developing in water for several hours.

In all these three sets of eggs, the first polar body was given off in the normal position and apparently in the normal manner before the eggs showed any signs of disintegration. No difference was noticed in the development of eggs which had been put into water and those which had been left in the body of the toad. It does not seem, therefore, that such unusual conditions interfere at all with the late maturation processes provided these processes have started before the normal conditions are changed. No attempt was made to fertilize these eggs artificially, as it has never been found possible to fertilize either the eggs of *Bufo* or of *Rana* until they have received the thick jelly-like membrane which is secreted around them in the oviducts.

In all cases the eggs were fixed in corrosive-acetic and stained with a combination stain of borax carmine and Lyon's blue as described in a previous paper (King, '10).

II. The Disintegration of the Germinal Vesicle.

I have already given in detail a description of the early stages in the breaking down of the germinal vesicle, and as this new material confirms but adds nothing to that description, it will be necessary to give only a brief account of the changes in the egg directly preceding the formation of the spindle.

At the end of the hibernation period the germinal vesicle lies in the upper hemisphere of the egg. It is round in outline and contains a large number of nucleoli which usually form a ring enclosing the chromatin threads. A layer of granular substance staining differently from the cytoplasm, surrounds the lower pole of the germinal vesicle and extends half way up each side. This substance appears homogeneous at first and then becomes a compact, fibrous band of uniform thickness. I have called this band a "line of radiation," because, as soon as the nuclear membrane has disappeared in this region, the karyoplasm of the nucleus forms into coarse granules and a pronounced radiation extends up into the nuclear substance from the entire length of the fibrous band below. The karyoplasmic granules soon become smaller and more numerous and finally disappear entirely, while the radiation from below continues to increase and often extends nearly to the upper surface of the egg. The rays forming this radiation are very fine, and their outer ends run, apparently, into the coarse network which comes to fill the entire space formally occupied by the germinal vesicle. During these changes, the nucleoli have lost their power of staining and have begun to disintegrate.

When the nuclear membrane breaks down, twenty-four chromosomes, arranged in pairs, are scattered throughout the upper part of the nuclear space. The ends of each pair then unite to form a closed ring near which a small aster usually appears. The aster has no centrosome and its rays rarely touch the chromatin ring. At the next stage, when the radiation from below has reached its greatest extent, the asters and the chromatin rings entirely disappear. Later, when the radiation has begun to decrease, a large number of small round chromatin granules are found near or on the line of radiation which has been gradually shortening during this period. When the chromatin granules first appear they stain very faintly, but they soon take the deep carmine stain characteristic of chromatin, and then fuse into several large, irregular clumps.

III. The Formation of the First Polar Spindle.

The line of radiation, shortly after the appearance of the chromatin granules, is shown in Fig. 1. It is a short, fibrous band with its ends, usually, though not invariably, slightly curved in towards the center of the egg. This structure, which is to become the first polar spindle, lies some distance below the surface of the egg in a small accumulation of granular substance formed, possibly, from the karyoplasm of the germinal vesicle. Its longitudinal axis may be either parallel or oblique to the surface of the egg, the latter position being the more common. Running out in every direction from the compact meshwork of fibers are numerous fine, thread-like rays which are longest and most numerous at the middle of the forming spindle where they extend out between the yolk spherules and seem to be continuous with the cytoplasmic network of the egg.

Collected near the middle of the spindle is a mass of small chromatin granules which are of uniform size and stain but faintly in comparison with the chromosomes of an earlier and of a later period. There is a very large number of these granules and it is quite impossible to count them satisfactorily; two other sections of the same egg each show as many granules as are shown in Fig. 1.

The nucleoli from the germinal vesicle appear at this period as irregular, yellowish green, refractive bodies which are scattered throughout the upper hemisphere of the egg, often lying quite close to the spindle. They disappear at different times in different eggs. Sometimes they have all been absorbed before the chromosomes have divided; sometimes they can still be found after the first polar body has been given off. I have never found any traces of them, however, after the spermatozoōn has entered the egg.

Not more than fifteen minutes after the stage of Fig. 1, the chromatin granules begin to fuse into irregular-shaped clumps. The number and size of these clumps vary greatly in different eggs, in some cases there are but four or five of them, in others at least twenty. Owing, probably, to their greater volume, these larger masses always stain much more intensely than do the small granules. Meanwhile the spindle has lost its uniform diameter and has become much thicker in the middle where the meshwork of fibers appears more distinct and more regular. The spindle soon becomes barrel-shaped and its fibers are quite clearly defined in the middle region but not at the poles (Fig. 2). The radiation from the spindle disappears entirely except at the poles where it forms distinct asters; some of the rays are very long and cross each other at the equator of the spindle. During its migration towards the upper pole of the egg the spindle shortens somewhat and gradually becomes more slender and pointed, a phenomenon seen by Van Name (17) in the eggs of Planarians, by Korschelt (12) in *Ophryotrocha*, by Griffin (8) in *Thalassema*, and by Boveri (1) in *Ascaris*.

At no stage in the formation of the spindle or in its later history can any centrosome be found in the polar asters. As the spindle becomes more pointed, the rays converge more sharply at the poles, but even when the radial systems are best developed (Figs. 2, 3), the rays appear to run into each other in the center of each aster and there is not the slightest trace of any kind of a central body. Carnoy and Lebrun (2) in their study of the batrachian egg, Eismond (5) in his work on *Siredon* and *Triton*, Fick (6) in studying the maturation of the Axolotl egg, and *Sobotta* (15, 16) in working on the egg of the mouse and of *Amphioxus*, have all failed to find a centrosome in the asters of the polar spindles. If such a structure is normal in these eggs and also in the egg of *Bufo lentiginosus*, methods of fixation and staining which have so clearly demonstrated its presence in other eggs are totally inadequate in these cases to show the slightest trace of it.

At the stage of Fig. 3, the small chromatin granules have entirely disappeared. Whether they have all gone into the large chromatin clumps or whether some have been absorbed by the cytoplasm cannot be determined. At this time the number of large chromatin masses still varies slightly in different eggs; in some cases there are nine such clumps of chromatin, in others at least fifteen. These chromatin masses are, for the most part, scattered irregularly along the spindle fibers, occasionally, however, one or more of them can be seen entirely outside of the spindle (Fig. 4, *CM*). Isolated masses of chromatin are sometimes found near the spindle at a much later period when the chromosomes are at the equator preparing to divide. They have entirely disappeared by the time the first polar body is given off, possibly serving as food for the cytoplasm as suggested by Gardiner (7).

During the next half-hour, the irregular chromatin masses change into chromosomes with a definite shape. The change does not take place at the same time in all of the chromatin clumps; in fact, until the chromosomes are arranged at the equator of the spindle ready to divide, they may be found in several different stages of development on the same spindle. Twelve chromosomes, one-half the number characteristic of the somatic cells of this species differentiate from the chromatin masses. The chromosomes are scattered over the entire spindle and are at first somewhat triangular in shape (Fig. 3), later they become rod-shaped structures which may lie with their long axis parallel, oblique, or even at right angles to the longitudinal axis of the spindle (Fig. 4). Sooner or later, however, the long axis of each chromosome comes to lie parallel with the spindle fibers and the chromosomes then have a rounded knob in the middle region and frequently also a smaller knob at each end (Figs. 4, 5). Later the middle knob becomes more prominent and the end knobs disappear (Fig. 5).

At the stage of Figs. 2–3 the asters at the spindle poles reach their greatest development. There are many long rays from each aster which run nearly parallel with the spindle fibers and cross each other at the equator of the spindle, and fewer and much shorter rays going out in other directions. Soon after this time the asters begin to degenerate. The shorter rays disappear first and by the time the spindle has reached the periphery of the egg there is not a trace of the radiation left. The spindle fibers then converge at the poles which are surrounded by a small accumulation of granular substance probably formed from the disintegrated rays (Fig. 7).

There is often a marked difference in the size of the chromosomes on the same spindle even when they are of exactly the same shape. One or two of the chromosomes may extend over one-third the length of the spindle, the others being not more than one-half as large (Fig. 5). This difference is not found at a later period; for, when the chromosomes are arranged at the equator of the spindle ready to divide, they are considerably smaller than the chromosomes of an earlier period and are all, apparently, of the same size.

While the chromosomes are being arranged at the equator of the spindle they undergo further changes in form. The polar arms shorten considerably, while the thick knob at the middle increases in size and gradually spreads out laterally, thus forming two wing-like projections on the chromosomes (Figs. 6, 7). In proportion as the lateral wings grow larger the polar arms of the chromosomes become shorter and thinner, so that there can be no question but that this lateral growth takes place at the expense of the rest of the chromosome. In a dorsal view, the wings appear to be spread out flat on the spindle and the chromosome has the appearance of a cross in which the polar arms are somewhat longer than the equatorial arms (Fig. 6). In a lateral view, however, the wings are seen to be raised up from the spindle while the polar arms are extended along the spindle fibers. Carnoy and Lebrun (2) have applied the term "oiselet" to this stage in the development of the chromosome. The typical oiselet stage is followed by one in which the body of the "bird" gradually disappears while the wings constantly increase in size (Fig. 7). Very soon, all that is left of the original polar arms is a slight projection on each side of the angle formed by the meeting of the two wings (Fig. 8). In the succeeding stage every trace of the polar arms has disappeared and there are twelve broad V-shaped chromosomes arranged at the equatorial plate with the angle of the V turned in towards the center of the spindle (Fig. 9). Usually, before this last stage is reached, the spindle has come to lie close to the surface of the egg and nearly radial in position. This is by no means invariably the case, however, as sometimes the spindle is still some distance below the surface of the egg when the chromosomes have divided in preparation for the first maturation division.

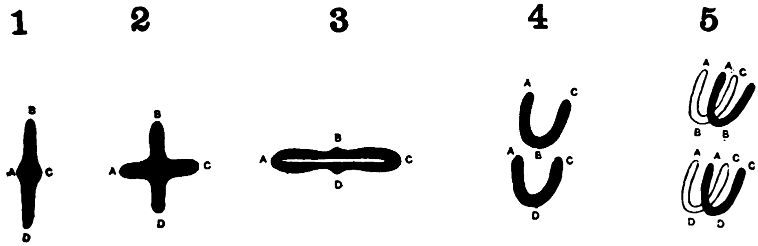
Fig. 6 shows part of a section of an egg fixed as soon as possible after the toad was killed. The spindle lies at the periphery of the egg and the chromosomes, with well-developed lateral wings, are at the equator. That this egg and others from same series are normal cannot be questioned. They show phenomena exactly similar to those seen in eggs that have been developing in water for some three hours, and leave no doubt but that the earlier processes described above are normal in spite of the unusual conditions to which many of the eggs were subjected.

Four V-shaped chromosomes in which all traces of the polar arms have disappeared are shown in Fig. 9. The arms of the V's are broad flat plates which form a sharp acute angle with each other. There is, in this case, no sign of a splitting in any of the chromosomes which are all of the same size and shape and arranged at the equatorial plate with the angle of the V turned in towards the center of the spindle, a characteristic arrangement of the chromosomes at this period. An equatorial section of a spindle in the same stage as Fig. 9, is seen in Fig. 10, where all twelve chromosomes are present. In this egg there are also found near the spindle a number of nucleoli which are in the process of disintegration.

Usually the first indication of any division of the chromosomes is seen at the stage of Fig. 11 when the polar arms have entirely disappeared and the chromosomes are broad V-shaped structures. At this time the ends of the V's often show a deep indentation (Fig. 11) indicating the longitudinal splitting of the chromosomes. Occasionally I have found the first division coming in at an earlier period before the entire disappearance of the polar arms. Such a division is seen in the chromosome at the left in Fig. 7. In all such cases the splitting is confined entirely to the lateral wings and never extends into the polar arms.

In the egg from which Fig. 12 was drawn, there are twenty-four V-shaped chromosomes which are similar to the twelve chromosomes in Fig. 10 in every respect except that they are much narrower. They have been produced, I believe, by a longitudinal division of the broad V-shaped chromosomes found at an earlier period. In some of the chromosomes shown in Fig. 12, the division for the second polar mitosis is seen. This second division of the chromosomes is not visible, at this stage, except in equatorial sections of the spindle. In longitudinal sections of the spindle the chromosomes always appear to be arranged in tetrad groups, one of which may be seen in Fig. 12. Such a group is, in reality, a pair of V-shaped chromosomes with the angle of each V turned in towards the center of the spindle, the four ends of a pair of chromosomes projecting from the spindle give the appearance of a typical tetrad. The maturation divisions of the chromosomes are represented diagrammatically by text-figures 1–5.

In my previous paper, three sections from one egg (Figs. 25, 26, 27) were given in which the fully formed spindle lay some distance below the surface of the egg and the chromosomes were in the form of closed rings which were split longitudinally. This egg was undoubtedly abnormal and led to the wrong inference that these chromatin rings were identical with those found in the germinal vesicle just previous to its disintegration. If the split V-shaped chromosomes of Fig. 11 were to be spread out in the form of a ring and the second maturation division to take place before the halves of the ring separated, then exactly the same effect would be produced as previously illustrated in Figs. 25–27. I can only interpret the ring-shaped chromosomes in this abnormal egg — the one abnormality I have found in many hundreds of eggs sectioned — as due to a delay in the separation of the parts after the two divisions of the chromosomes had taken place.



Diagrams of the maturation divisions of the chromosomes in the egg of Bufo lentiginosus.

According to Carnoy and Lebrun (2, 3) who have published a series of memoirs dealing with the development of the germinal vesicle and the formation of the polar bodies in the eggs of various Batrachia, the chromatin filaments in the egg of Salamander, *Alytes*, *Triton*, *Bufo* and *Rana* arise from repeated resolutions of the nucleoli in the germinal vesicle. As my own work on *Bufo* began with the fully formed egg taken from the animal just before the beginning of the hibernation period, I have not yet seen the resolution of the nucleoli into chromatin threads. In all the eggs that I have examined in which the germinal vesicle was intact, the chromatin was always in the form of distinct chromosomes. These chromosomes had no connection whatever with the large round nucleoli which, with the combination stain used, always stain a deep blue while the chromatin invariably takes the carmine. I have frequently noticed, however, that many of the chromosomes end in small granules which take the same stain as the chromatin and that there are a number of similar granules scattered throughout the nucleus. Recent work on various forms has shown that unquestionably the term nucleolus has been applied to many different kinds of structures in the germinal vesicle. As a general term used to cover any definite structures in the germinal vesicle other than chromosomes, linin and karyoplasm, it may, perhaps, be fitly applied both to the large rounded structures (which I consider the only true nucleoli in the germinal vesicle) and to the smaller granules which stain like chromatin and which I believe to be chromatin that is not used for the chromosomes. The structures which, in my opinion, are the true nucleoli have nothing to do with the formation of the chromosomes for the first polar spindle, as they are never connected with the chromosomes in any way and can be traced step by step until they are absorbed by the cytoplasm of the egg after the spindle is completely formed.

Many of Carnoy and Lebrun's illustrations of the formation of the first polar spindle in the egg of *Bufo vulgaris* are strikingly like my own, but we differ somewhat in our interpretation of them. According to their view, when the germinal vesicle in the egg of *Bufo vulgaris* migrates towards the upper pole, and before the nuclear membrane disappears, the paired chromatin filaments (which are exactly like those I find in *Bufo lentiginosus* during the same period) break up into small granules which cannot be distinguished from the granules of karyoplasm. *All the nucleoli suffer the same fate as the chromosomes excepting about ten which remain to form the chromosomes of the first polar spindle.* The karyoplasm meanwhile, forms a pronounced radiation from the "plage fusoriale" at the lower pole of the germinal vesicle. "Les nucléoles prédestinés montent le long des filaments" and are carried to the "plage fusoriale" where they either become vacuolated in the center and form a ring, or else they fuse into one large mass and later regain their individuality. When the spindle is first formed, the chromosomes are very irregular in shape and there are distinct asters at the spindle poles which never contain a centrosome.

In the egg of *Bufo lentiginosus*, I have traced the chromosomes of the germinal vesicle up to the stage where the ends of a pair of chromosomes unite to form a closed ring. After this time, although I have had an abundance of material and have searched very carefully through every section of the germinal vesicle in a large number of eggs, I have been unable to find any trace of the chromatin. There is, I believe, no doubt but that the chromatin rings break up into minute granules which may, possibly, be carried by the karyoplasmic radiation to the lower pole of the germinal vesicle where they later form the chromosomes of the first polar spindle. I have never seen anything in this egg, however, that would indicate that some of the *nucleoli* are destined to form the chromosomes of the first polar spindle. A large number of nucleoli are always present throughout the early stages of maturation and they all appear to be undergoing the same processes of disintegration. Carnoy and Lebrun might consider the large irregular masses shown in Fig. 2 to be nucleoli in the general sense in which they seem to use the word, but these masses have been formed by the fusion of smaller chromatin granules (Fig. 1) and are in no way connected with the true nucleoli of the germinal vesicle.

Carnoy and Lebrun have followed the details of the formation of the first polar spindle and the later changes of the chromosomes much more carefully in the egg of *Triton* than in any of the other amphibian eggs they have studied. Their account of this form agrees substantially with that of *Bufo vulgaris* as regards the breaking down of the germinal vesicle, with the important exception that in *Triton*, *all* of the nucleoli are absorbed by the cytoplasm, none of them are reserved, as in *Bufo Vulgaris*, to form the chromosomes of the first polar spindle. The chromatin threads which were resolved from the nucleoli at an earlier period, break up into very small granules when the membrane of the germinal vesicle disappears. The twelve chromosomes which later arise *from a coalescence of the chromatin granules* are at first very irregular in shape and they are scattered all along the spindle fibers; subsequently they undergo a double longitudinal division in preparation for the giving off of the polar bodies. Any chromatin not used for the chromosomes is absorbed by the cytoplasm.

Although there are always twelve chromosomes on the first polar spindle in the egg of *Triton*, Carnoy and Lebrun find only 8–10 chromosomes in the equatorial plate of the first polar spindle in the egg of *Bufo vulgaris*, and but 4–5 chromosomes at each pole just previous to the giving off of the first polar body. The failure of these investigators to find the definite number of chromosomes that must be present unless the egg of *Bufo vulgaris* is a marked exception to the rule that the number of chromosomes is constant for a given species, may possibly be accounted for on the supposition that some of the chromosomes were lost when the eggs were sectioned or that the sections of the egg were made so thick that some of the chromosomes were not visible.

In a more recent paper, Lebrun (13) gives the results of a reexamination of the maturation processes in the egg of *Triton*. He states that the double longitudinal division of the chromosomes does not take place in the complicated manner previously described by Carnoy and Lebrun, but according to the scheme represented by my text-figures 1–5. The late maturation changes in the egg of *Triton* are, therefore, strikingly similar to those I have found taking place in the egg of *Bufo lentiginosus*. Lebrun still believes that in the eggs of *Rana temporaria* and of *Bufo vulgaris* a certain number of the nucleoli are reserved to form the chromosomes of the first maturation spindle. A reëxamination of the maturation stages in the eggs of these amphibians would probably show that in these forms also the chromosomes are derived from fused masses of chromatin granules and that they have no connection whatever with the true nucleoli.

I have examined a large number of the eggs of *Bufo* at the stages of Figs. 3–4 and I can see no reason for believing with Carnoy and Lebrun that a division of the chromosomes place at this time. During this period the chromosomes are exceedingly varied in size and shape. If the chromosome is oblong, it may have either its long or its short axis parallel with the longitudinal axis of the spindle; if the chromosome is pyramidal in shape, either the base or the apex of the pyramid may rest on the spindle fibers. I regard all of the changes in the shape of the chromosomes up to the stage of Fig. 7 as due solely to a rearrangement of the chromatin material preparatory to the later divisions. The first indication of any division of the chromosomes is the longitudinal splitting of the lateral wings which in some few cases can be found before the disappearance of the polar arms (Fig. 7). The apparent separation of the lateral wings at *X*, Fig. 11, I consider to be due to the fact that the angle of the V-shaped chromosome was cut off in sectioning. It very frequently happens that portions of one or of several chromosomes on a spindle are removed in this way. Sometimes, as in Fig. 4, the median knob of a chromosome is lacking; sometimes, the lateral wings have been removed (Figs. 6, 7). In rare instances the cut off portion of the chromosome will be found in the next section of the egg; but as the chromosomes are quite small a careful examination of the following sections often fails to disclose the missing part.

As found to be the case in many eggs besides that of *Bufo*, for example in *Cerebratulus* (Coe), *Polychœrus caudatus* (Gardiner), *Thalassema* and *Zirphœa* (Griffin), and *Triton* (Carnoy and Lebrun), all the chromatin of the germinal vesicle does not go to form the chromosomes of the first polar spindle, some of it is thrown out into the cytoplasm where it degenerates and sooner or later completely disappears. Even in the segmentation stages of the egg of *Ascaris*, Boveri (1) found that some of the chromatin is thrown out of the nucleus and absorbed by the cytoplasm. In all these cases there is obviously a mass reduction of the chromatin in preparation for the succeeding division of the cell. It may be, as suggested by Gardiner, that "there are two kinds of chromatin stuff, the one insoluble and bearing the heredity which is to be transmitted to the daughter cells, and the other food for the cytoplasm." This theory would explain the facts as we now know them, but it cannot be proved until some stain can be found to differentiate the two sorts from each other.

Carnoy and Lebrun find a double division of the chromosomes in the egg of *Triton*, and they state that there is no reason why a longitudinal division of the chromosomes should not be a reduction division in the Weismann sense, in that it may separate the chromosome into two parts each containing different kinds of granules: it is certainly true if we admit a difference in the properties of the elementary granules. As all of the chromatin granules do not go into the chromosomes of the first polar spindle, there is a process of selection in the formation of the chromosomes and their subsequent division would be a permanent source of variation for the descendents.

The chromosomes of the first polar spindle in the egg of *Bufo lentiginosus* are at first exceedingly varied in shape; they may be round, triangular, or oblong. At this time it is obvious that they have no definite longitudinal axis. At the stage of Fig. 5 the chromosomes have elongated and lie parallel with the longitudinal axis of the spindle. When the wings have formed, there is a stage when the arms of the chromosomes are all approximately of the same length (Fig. 6). Is there a definite longitudinal axis at this time? If the part of the chromosome resting upon the spindle fibers is considered to be the longitudinal axis, then later this same axis is not only shorter than the transverse axis, but it practically disappears at the stage of Fig. 9. If shown Fig. 9 without the preceding figures, no one, I am sure would call the thickness of the chromosome at the angle of the V the longitudinal axis of the chromosome, and the division indicated in Fig. 11 would unhesitatingly be called a longitudinal division. If one arbitrarily states that the polar arms of the chromosomes in Fig. 5 form the true longitudinal axis, not only in this particular stage, but until division is completed, then the splitting seen in Fig. 11 is a transverse division, as is also the second division which takes place in the same direction. On the other hand, if the longer axis of the chromosome at the time when division occurs is considered to be the true longitudinal axis, then there is a double longitudinal division of the chromosomes and the egg of *Bufo* is thus brought into line with other amphibian eggs that have been studied. It would seem, as suggested by Sebaschnikoff (14), that the distinction between transverse and longitudinal divisions of the chromosomes is not as important as many investigators have claimed: the division of the chromatin substance would appear to be the important thing, the manner of its achievement quite secondary, as Hertwig (9) has maintained.

There is, however, the following possibility to be considered. When the germinal vesicle breaks down, all of the chromosomes are arranged in pairs, in some cases the ends of a pair of chromosomes have united to form a closed ring. Very soon after this stage the chromosomes break up into granules and all traces of the chromatin substance is lost until innumerable chromatin granules appear in connection with the first polar spindle. It is conceivable that all of the chromatin granules belonging to a pair of chromosomes have remained united during this period of the apparent disintegration of the chromosomes, although I have not been able as yet to demonstrate such a union. If such is the case, then the chromosomes of the first polar spindle are bivalent structures, each being composed of the two chromosomes that had become paired at an earlier period of development. On this assumption it is probable that the knob-like thickening in the middle of the chromosomes, shown in Figs. 4 and 5, is caused by the fusion of the ends of the two chromosomes. In text-figure 1, *ABC* and *ACD* would represent the two chromosomes united at *AC*. The subsequent changes in the shape of the chromosomes serve merely to again elongate the original chromosomes (Text-fig. 3) which are finally separated by the division through *AC*. The first maturation division, therefore, is a reduction division and the second division only is a longitudinal one. It certainly cannot be mere chance that at the time of the breaking down of the germinal vesicle, the chromosomes should invariably become arranged in pairs. In light of the most recent investigations on spermatogenesis and ovogenesis it would seem as if the above explanation must be the true one for the maturation divisions in the egg of *Bufo*, although at present I am not able to prove it. I hope that the work I am doing on the spermatogenesis of this amphibian will throw some light on the maturation divisions in the egg.

LITERATURE.

1. **Boveri, Th.**
'87 Zellen-Studien, Heft I. Die Bildung der Richtungskörper bei Ascaris megalocephala und Ascaris lumbricoides. Jena, 1887.

2. **Carnoy, J. B., et Lebrun, H.**
'99 La vésicle germinative et les globules polaires chez les Batraciens. La Cellule, t. XVI., 1899.

3. **Carnoy, J. B., et Lebrun, H.**
'00 La vésicle germinative et les globules polaires chez les Batraciens, Deuxieme Partie, Les Anoures. La Cellule, t. XVII., 1900.

4. **Coe, W. R.**
'99 The Maturation and Fertilization of the Egg of Cerebratulus. Zool. Jahrbüchern, Bd. XII., 1899.

5. **Eismond, J.**
'94 Einige Beiträge zur Kenntniss der Attractionssphären und der Centrosomen. Anat. Anz., Bd. X., 1894.

6. **Fick, R.**
'93 Ueber die Reifung und Befruchtung des Axolotleies. Zeitsch. f. wiss. Zool., Bd. LVI., 1893.

7. **Gardiner, E. G.**
'98 The Growth of the Ovum, Formation of the Polar Bodies, and the Fertilization in Polychœrus caudatus. Journ. of Morph., Vol. XV., 1898.

8. **Griffin, B. B.**
'99 Studies on the Maturation, Fertilization and Cleavage of Thalassema and Zirphæa. Journ. of Morph., Vol. XV., 1899.

9. **Hertwig, O.**
'90 Vergleich der Ei- und Samenbildung bei Nematoden. Arch. f. mik. Anat., Bd. XXXVI., 1890.

10. **King, H. D.**
'00 The Maturation and Fertilization of the Egg of Bufo lentiginosus. Journ. of Morph., Vol. XVII., 1900.

11. **King, H. D.**
'02 Preliminary Note on the Formation of the First Polar Spindle in the Egg of Bufo lentiginosus. Anat. Anz., Bd. XXI., 1902.

12. **Korschelt, E.**
'95 Über Kerntheilung, Eireifung und Befruchtung bei Ophryotrocha puerilis. Zeit. f. wiss. Zool., Bd. LX., 1895.

13. **Lebrun, H.**
'02 La vésicule germinative et les globules polaires chez les Anoures. La cellule, T. XIX., 1902.

14. **Sabaschnikoff, M.**
'97 Beiträge zur Kenntniss der Chromatinreduction in der Ovogenese von Ascaris magalocephala bivalens. Bull. de la Soc. impériale des Naturalistes de Moscow, 1897.

15. **Sobotta, J.**
'95 Die Befruchtung und Furchtung des Eies der Maus. Archiv. f. mik. Anat., Bd. XLV., 1895.

16. **Sobotta, J.**
'97 Die Reifung und Befruchtung des Eies von Amphioxus lanceolatus. Arch. f. mik. Anat., Bd. L., 1897.

17. **Van Name, W. G.**
'99 The Maturation, Fertilization, and Early Development of the Planarians. Trans. of the Conn. Acad., Vol. X., 1899.

EXPLANATION OF PLATE.

All figures were drawn with the aid of a camera lucida under a Zeiss Apoc. 2 mm., Oc. 4.

1. An early stage in the formation of the first polar spindle before the chromatin granules have fused into large masses.

2. A stage about one-half hour later than Fig. 1. The spindle has become barrel-shaped and the chromatin granules are fusing into large masses to form the chromosomes.

3. Twelve irregularly shaped chromosomes have differentiated from the chromatin masses and lie scattered along the spindle.

4. Spindle parallel to the surface of the egg. The chromosomes have elongated and many of them show a median knob. *C.M.*, chromatin mass outside the spindle.

5. About the same stage as Fig. 4. Chromosomes of very different sizes are found on the spindle.

6. Typical "oiselet" stage.

7. Chromosomes in various stages of development on the same spindle. In some of the chromosomes the splitting for the first maturation division can be seen while the polar arms are still present.

8. Section showing the growth of the lateral arms of the chromosomes at the expense of the polar arms.

9. The V-shaped chromosomes after the disappearance of the polar arms.

10. An equatorial section of a spindle at the stage of Fig. 9. All twelve chromosomes are present.

11. Equatorial section. The notched ends of some of the chromosomes indicate the direction of the first maturation division.

12. Equatorial section. The first maturation division is completed and the second maturation division is indicated in some cases.

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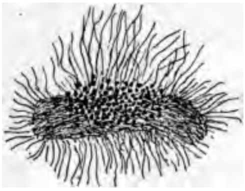

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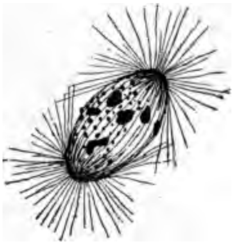

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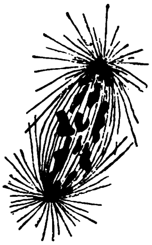

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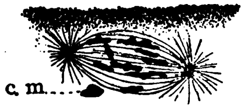

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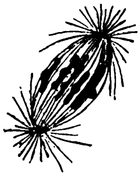

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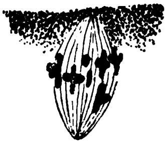

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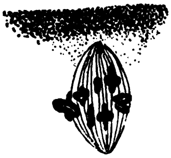

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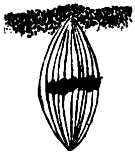

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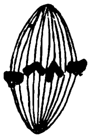

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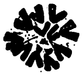

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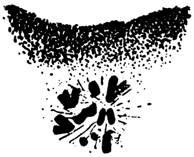

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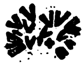

From the Biological Laboratory of Bryn Mawr College.

A STUDY OF THE GERM CELLS OF APHIS ROSÆ AND APHIS ŒNOTHERÆ.[1]

BY

N. M. STEVENS.

Associate in Experimental Morphology, Bryn Mawr College, and Research Assistant, Carnegie Institution of Washington.

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This work was undertaken in connection with a series of experiments on sex-determination in Aphids. On approaching the subject, it was found that very little is known concerning the ovogenesis and spermatogenesis of these insects. Blochmann ('87) showed that the parthenogenetic egg gives off one polar body, and the winter egg two; but I find no account of the number of chromosomes or the method of reduction in either egg, nor is there any published work on the spermatogenesis of Aphids. The present paper will be devoted mainly to the germ-cells, a few points in the early stages of parthenogenetic development being considered incidentally.

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[1] This paper was awarded the prize of one thousand dollars offered in 1904 by the "Association for Maintaining the American Woman's Table at the Zoölogical Station at Naples, and for Promoting Scientific Research among Women."

I. MATERIAL AND METHODS.

Sections of Aphids from various host plants—the rose, English ivy, honeysuckle, alder, arrow-head, Œnothera, and hop, were examined; but Aphis rosæ proved the most satisfactory for study of the ovogenesis of both parthenogenetic and winter eggs, and Aphis œnotheræ for the spermatogenesis.

Both the Aphids and the winter eggs were killed and fixed with Gilson's aceto-chloroform-sublimate mixture, which gives far better results than any other method tried. Very small Aphids were embedded whole; larger ones after removing the head and thorax. Sections were cut from 5 μ to 7 μ thick, and stained with Delafield's hæmatoxylin and orange G, or with Heidenhain's iron-hæmatoxylin. The former method of staining gives remarkably good differentiation for ordinary purposes. The latter is more satisfactory for the study of ovogenesis and spermatogenesis. Many slides stained at first with Delafield and orange, were re-stained with iron-hæmatoxylin for more careful study.

II. PARTHENOGENETIC DEVELOPMENT.

1. Female Line.

Most of the stages in ovogenesis of parthenogenetic eggs and also early segmentation stages were obtained from sections of the unborn young of the viviparous female. For more advanced segmentation stages and early embryos very young Aphids were sectioned.

All the species examined have twelve ovaries in two groups as described by Balbiani ('69–'72). Oögonial mitoses were observed, but offered nothing of especial interest as the division figures are identical with other embryonic mitoses.

The resting oöcyte, before the growth stage of the ovum begins, has a very large nucleus in proportion to the size of the cell (Figs. 1 and 2). A large nucleolus occupies the center of the nucleus, and in addition to this the nucleus contains a linin reticulum and irregular masses of material, presumably chromatin, which does not take the hæmatoxylin stain at this stage.

One at a time the oöcytes at the posterior end of the ovary enlarge and push out into the oviduct, remaining, however, connected by a stalk with the central core of the ovary (Fig. 1). As many as three eggs may be thus connected by stalks with the center of an ovary,—one egg just passing into the oviduct, another in maturation stage, and a third in 8 to 16-cell stage. As the oöcyte increases in size, the cytoplasm changes its staining reaction, coloring somewhat deeply with Delafield; and the nucleus shows various changes. The nucleolus disappears and the chromosomes become stainable at an early stage (Figs. 1, 3, 4, 5). As the egg rapidly enlarges the nucleus approaches the periphery at one side of the oval egg (Fig. 6). Just before maturation, clear vesicles appear in the cytoplasm and soon fuse forming large irregular spaces filled with a clear non-staining substance, presumably yolk material, separated from the general cytoplasm (Blochmann).

Fig. 7 shows the equatorial plate of a polar spindle in metaphase. There are 10 chromosomes of 5 different sizes. This is the somatic number, and there is therefore no synapsis or conjugation of chromosomes and no reduction in the maturation of the female parthenogenetic egg. The same number (10) and the same variation in size (5 pairs) is shown in the segmentation spindles and equatorial plate of Fig. 12. The maturation spindle in anaphase is well shown in Fig. 8; also the lacunar spaces in the interior of the egg.

The polar body is at first completely extruded from the egg and separated from it as seen in Figs. 8 and 9. It is distinctly a polar body not a "polar nucleus." Soon, however, it comes to lie within the boundary of the egg among the segmentation nuclei (Figs. 10 and 11). In such a stage as in Fig. 11, the polar body is easily recognizable by its clear cytoplasm and deeply-staining mass of chromatin. It lies in a sort of vacuole in the egg-cytoplasm. I have been able to follow the polar body as far as the stage shown in Fig. 13. The cytoplasm can no longer be distinguished from that of the egg; the chromatin mass is irregular in outline, usually stains less deeply and appears to be degenerating. I therefore feel sure that the polar body takes no part in the development of the embryo. Its inclusion within the egg is probably due merely to mechanical conditions, *i. e.*, to the pressure of the walls of the oviduct upon it, as it lies on the side of the oval egg.

There is only one point of especial interest in the later development, and that is the relation of the young embryo to the vitellaria.

This is shown in Figs. 14 and 15. Fig. 14 shows an embryo which has just begun to take in yolk (the "secundäre Dotter" of Will, '89). At the base of the embryo as figured are two conspicuous cells (*b*) which apparently guard a valvular opening in the wall of the oviduct, and recall the four guard-cells at the inner end of the embryonic pharynx of Planaria simplicissima. At the lower focus of the section the valve is slightly open and a small amount of yolk material has entered (Fig. 14, *a*). In Fig. 15 the valve is widely open and yolk cells are being taken into the embryo. Whether the embryo actively sucks in the yolk, or the yolk cells themselves are the active agents, it is impossible to tell, but the former seems more likely. Will ('89) describes the secondary yolk as forming in connection with the follicle epithelium and then being taken into the gastrula. In speaking of the work of Witlaczil ('84), he says, "Von seiner ganzen Darstellung ist nur das eine richtig, das der secundäre Dotter von Follikelepithel seinen Ursprung nimmt," and later he says, "Diese innerhalb der Epithelverdickung producirte Dottermasse ist es nun welche in das Ei eintritt und demselben den sogenannten secundären Dotter liefert. Dass es sich dabei nicht um eine Einwanderung von Zellen . . ."

The close resemblance in structure between the secondary yolk and certain large cells in the body cavity of the mother was easily observed in the sections, but the relation between the embryo and the vitellaria described above was first seen in connection with the egg of Aleurodes, a related form, where the relation is much more conspicuous than in the Aphid. It was later traced with certainty in the winter egg and in the parthenogenetic embryo of the Aphid.

No variation could be detected in the development of ova which produce winged parthenogenetic individuals. The winged young can often be distinguished before birth, and some of the same brood may be winged, others apterous. The winged parthenogenetic individuals are migrants and their appearance seems to be conditioned by the amount or the quality of the food supply.

2. *Male Line.*

The mothers of the males are apterous and cannot be distinguished externally from the apterous females which produce female offspring. The ovaries show no difference in structure, unless it may be in size and number of the oöcytes, and only one polar body is given off. In studying the development of male[1] eggs, one is at a disadvantage so far as the amount of available material is concerned, because only eggs connected with the ovaries of females which contain embryos large enough to be recognized as male can be utilized, while in the female line the best eggs for study are found in abundance in the larger embryos. No polar spindles were found in the few male eggs obtained. Figs. 17 and 18 show the polar body as it appears in eggs containing 4 and 8 nuclei. There is no indication of fusion of two polar bodies. In Fig. 18 the chromosomes are not so fully fused as in Fig. 17, but this is the condition at a corresponding stage in the female eggs. Examination of the segmentation spindles in male embryos and of spermatogonial mitoses makes it certain that the full somatic number of chromosomes is present until we come to the spermatocytes when reduction occurs. This is what we should expect had it not been claimed by Castle ('03) that the female character which is usually dominant in parthenogenetic insects is removed from the egg with the second polar body, thus allowing the recessive male element to assert itself. There is no evidence in my material of any difference between the maturation of the female parthenogenetic egg and that of the male egg, and until I can procure more material and examine the point further, I shall assume that the method of maturation of all parthenogenetic Aphid eggs is the same, *i. e.*, only one polar body is produced and there is no reduction of chromosomes. There is no mixture of male and female young in the offspring of one individual: certain apterous females produce only females, either winged or apterous, and others produce only winged males.

The ovarian oöcytes, eggs and polar bodies in the male line (Figs. 16, 17, 18) are noticeably larger than those figured in the female line (Figs. 1–12), but this may be due to the fact that the drawings were made from different species; the former from Aphis œnotheræ, the latter from Aphis rosæ. The difference is one of size not of structure, and much the best and most abundant male material was obtained from Aphis œnotheræ, where parthenogenetic reproduction was wholly replaced by the sexual method early in October, and young males of all ages were abundant.

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[1] Male is used here merely in the sense that these eggs produce males.

III. THE WINTER EGGS.

1. Early Development and Growth.

The material for study of the winter eggs was obtained by bringing into the laboratory rose twigs with broods of sexual females on the leaves. Adult males were usually found on the same leaves. These young females develop more rapidly in a warm room under glass, and soon begin to lay the fertilized eggs. The winged mothers of the sexual females were sectioned for the study of the ovaries of the sexual female embryos, and young sexual females for early stages in the development of the winter egg. Fig. 19 shows an ovary from such an embryo. It is considerably larger than the ovary of the ordinary parthenogenetic female embryo (Fig. 1), and in every case these ovaries show a large number of degenerating oöcytes at the posterior end (Fig. 19, *a*). These cells are more or less shriveled and stain deeply and irregularly. Whether this is simply degeneration of a large number—at least half—of the oöcytes of an ordinary parthenogenetic ovary in order that the remainder may have room for growth, or whether the parthenogenetic ovary may contain originally both eggs capable of parthenogenetic development and others capable of development only after fertilization, and the former degenerate in order that the latter may develop, I am unable to say. The number of oöcytes in the twelve parthenogenetic ovaries is certainly more than double the number of young ever produced by an individual, so that the latter supposition might be possible. Two cases observed in dissection, but never duplicated in fixed material, might support either view, and certainly tend to show that the ovary which produces parthenogenetic embryos and the ovary which produces winter eggs are originally identical. In two individuals egg-strings and winter ovaries with developing eggs were found associated in both groups of ovaries. The number in each varied—in one individual one group contained 5 egg-strings and one winter ovary, the other group 3 egg-strings and 3 winter ovaries; in the second individual each group contained 5 winter ovaries and one egg-string; on one side there was one parthenogenetic ovary with eggs and embryos (Fig. 20, *c* and *e*), on the other side was an egg-string consisting of two parthenogenetic embryos, a winter ovary and a young winter egg (Fig. 21, *a*, *b* and *e*). These individuals with mixed ovaries were found in the greenhouse January 23, 1904, on a small rose bush which had lost its leaves and was nearly dead as a result of serving as a food plant for several generations of Aphids. They were the third generation from a winged female. Two or three others escaped dissection. Unfortunately only freehand sketches were made, and no other such cases were observed. One of these sketches is reproduced in Fig. 20 and a part of another in Fig. 21. Similar observations were recorded by Bonnet ('45) and by Leydig ('50), and Kyber ('15) observed sexual forms on the willow in June and on ripening grain in midsummer, but did not connect both parthenogenetic young and winter eggs with the same individual.

The oöcytes in the winter ovaries increase immensely in size before any eggs are given off, and the large nearly spherical ovaries are easily distinguished in dissections from the minute oval parthenogenetic ovaries (Fig. 20, *a* and *c*). The first egg is given off after the birth of the sexual female.

It is not my purpose to describe the growth period of the winter egg in detail. The nucleus, at first central, gradually moves to the periphery at one side, usually nearer the anterior end of the egg as it lies in the oviduct. When the egg has reached about one-half its ultimate size, yolk material from the vitellaria is taken in at the posterior end of the egg. There seems to be no such definite opening as in the case of the parthenogenetic embryo and of the egg of Aleurodes. Fig. 22 is an oblique section through an egg which plainly shows the relation between the yolk within and that without the egg. Entrance seems to be effected between any of the cells of the follicle epithelium, which is merely a part of the oviduct. Here again one wonders which is the active agent, the egg or the yolk cells, but it is impossible to tell. In the case of the embryo one is inclined to believe that it may actively suck in the yolk as the planarian embryo does, but there is nothing to indicate that the egg could have any such power. In the case of Hydatina senta, Lenssen ('98) describes the yolk cells as penetrating the egg by their own activity. The entrance of yolk seems to be associated with a definite size of the ovum and would therefore occur at a definite point in the oviduct. The relation between the oviduct and the yolk gland may therefore be a more definite one than the sections show. Entrance of yolk must be effected very quickly as cases where the process can be demonstrated are very rare; and one finds no such intermediate stages as would necessarily appear if this secondary yolk were formed within the egg as described by Balbiani ('69–'72). The outlines of the yolk cells are lost and only fragments of nuclei are found (Fig. 22), while in Aleurodes several whole yolk cells with nuclei intact enter the oviduct below the egg and are later included in the posterior end of the egg.

The chromatin in the egg during its growth period offers no favorable conditions for study. In earlier stages it does not take chromatin stains, and in later stages the chromosomes are spherical and mingled with nucleoli of similar form and staining qualities.

2. *Maturation.*

The earliest stage found in the laid egg was that shown in Fig. 23—the equatorial plate of the first polar spindle, showing 5 chromosomes, the reduced number, of the same relative form and size as the chromosomes of the 5 pairs in Figs. 7 and 12. The manner in which the egg chromosomes are paired is not evident, but the two divisions appear to be longitudinal and identical with the maturation divisions of the spermatocyte where it is quite certain that they are paired longitudinally, and probable that the first division separates the paired chromosomes.

Fig. 24 shows the first maturation spindle in metaphase. One chromosome appears in both figures (stippled in *b*). In Fig. 25 the first polar body and the second polar spindle in metaphase are figured. A part of a chromosome appears in *a* and parts of two in the spindle of *b*. In Fig. 26, a later stage is seen; the chromosomes of the first polar body are massed together, those of the second (not yet separated from the egg) show the comparative size relation of Fig. 23, their position indicating a longitudinal division; the chromatin of the egg nucleus is becoming diffuse and less stainable.

The spermatozoön enters at any point, more often near the posterior end of the egg, and leaves a train of cytoplasm behind it, as it traverses the yolk preceded by an aster. Fig. 27 shows the male and female pronuclei, the former distinguished by the cytoplasmic path (indicated by arrows). I have never found the first segmentation spindle in my material nor indeed the divisions immediately following, though the resting nuclei of these stages have frequently been observed. In the later segmentation stages it is impossible to distinguish the individual chromosomes in the spindle; they are crowded together and often are so united in metaphase as to resemble a spireme in one plane.

IV. SPERMATOGENESIS.

Nearly all of the material for the study of the spermatogenesis was obtained from the Œnothera. On the rose the young males are never met with in large numbers while on the Œnothera an abundance of all sizes may often be found on the still-blossoming tips of the flower spikes, while the sexual females are scattered over the leaves and stalks. Here also were found a few mothers of the males. The few young males from the rose showed the same number and relative size of chromosomes.

The testes, as seen in dissections, have 6 lobes corresponding to the 6 ovaries in each group. The lobes are much larger than the ovaries, and as the spermatogonia are somewhat smaller than the oögonia, the number of mitoses leading to the spermatocyte must be several more than in the case of the oögonia. Only spermatogonial divisions are found in the embryos, and the last such division often, if not always, occurs after birth. Fig. 28 shows a resting spermatogonium; Figs. 29 and 30 spermatogonia just before division. In Fig. 30, 9 chromosomes of characteristic form and size can be recognized, one of the smallest not being visible. The equatorial plate, as also in most somatic mitoses, is too crowded for distinguishing either number or form of chromosomes.

The resting spermatocyte of the first order (Fig. 32) does not differ materially in appearance from the resting spermatogonium (Fig. 28) and indeed can often be recognized only by its relation to later stages. There is no evidence of so long a growth stage as in most forms. Closely associated with the first maturation mitosis and probably immediately preceding it are found the stages shown in Figs. 33 and 34. In Fig. 33, *a*, 8 of the 10 chromosomes are to be seen scattered through the nucleus; in *b*, *c* and *d*, chromosomes of the same form and size are seen paired longitudinally. Fig. 34 suggests the synapsis stage described for many insects. This stage is of much more frequent occurrence than that shown in Fig. 33, and appears to follow that, and immediately precede the first spermatocyte division which is shown in Figs. 35–39. Fig. 35, *a*, *b* and *c* show the 5 chromosomes in the equatorial plate of the first maturation spindle. All are connected by linin threads, and every possible arrangement of the 5 chromosomes is found in different cells. In *c* the longest chromosome is seen to be double and as a side view of the spindle in metaphase always shows the chromosomes double, it is probable that they come into the mitotic figure in that condition from the preceding conjugation stage. A second longitudinal split to form a tetrad cannot be detected at this stage. In Fig. 36, a side view and an oblique view of the equatorial plate show the double chromosomes. Two stages of the anaphase appear in Figs. 37 and 38. There is always one "lagging" chromosome in this division, and it certainly is not usually the longest, as I at first thought likely. In fact it appears in most cases to be either the second or third in size. After the two spermatocytes of the second order are fully formed, as shown in Fig. 39, the two daughter elements of this "lagging" chromosome are still connected by a thread extending through the cytoplasm of each cell. This phenomenon seems to be a peculiar characteristic of one of the chromosomes in this particular division. I have never seen an exception, nor have I ever seen anything similar in the second spermatocyte division or in fact in any other mitosis.

If, as I have supposed, this first maturation division simply separates paired chromosomes, it is possible that the pair that shows this peculiarity has a different linin connection from the others. In Fig. 35, it will be seen that the 5 chromosomes are united by linin threads into a chain with free ends. A side view (Fig. 36) shows two pairs connected by two parallel linin threads. Now if one of the end pairs always has its two elements connected by a single thread, we might expect such figures as 37, 38 and 39.

Fig. 40 is the equatorial plate of the second maturation mitosis, showing again the 5 chromosomes of characteristic form and size. Fig. 41 shows one of a few fortunate sections showing the daughter chromosomes in anaphase, and removing all doubt as to the kind of division, longitudinal or transverse.

Judging from analogy in other insects, I fully expected to find one longitudinal and one transverse division, but was soon convinced that both are longitudinal, and from such figures as 23 and 26 that the same is true in the maturation division of the winter egg. This point will be more fully discussed later.

The chromosomes retain their individuality in the spermatids for some time, but finally become massed together to form the sperm-head (Fig. 42). The development of the spermatozoön from the spermatid appears to be very simple, the head being formed mainly from the chromatin and the long, rather thick tail from the cytoplasm. None of the accessory structures described for other insects are present. No centrosome has been detected in any mitosis, and asters have been seen only in connection with the sperm-nucleus in the egg, the pronuclei, and the segmentation spindles of the winter egg. There is no trace of anything that could be called an "accessory chromosome" (McClung, '02). The nucleolus appears to be of the same character throughout, appearing in resting cells and disappearing in mitosis. That it is not a chromatin nucleolus, or karyosome, is shown by the fact that the chromosomes in many cases are visible before it disappears (Fig. 29) and with the Delafield-orange combination the nucleolus invariably takes the orange stain while the nuclear reticulum stains with the hæmatoxylin.

V. GENERAL DISCUSSION.

1. Mendel's Law, and the Individuality of the Chromosomes.

It appears that in the Aphids studied there is a series of 5 chromosomes of different shape and size in the germ cells of the sexual generation: the maternal or egg-series is exactly equivalent to the paternal or sperm-series. The chromosomes show the same relative form and size throughout the maturation divisions. The two series of chromosomes meet in fertilization, and throughout the parthenogenetic generations, both female and male, we find the double series, 10 chromosomes of five different sizes. In the spermatocytes, and presumably in the oöcytes, the chromosomes of the double series are paired, and in one of the maturation divisions, apparently the first, the paired chromosomes are separated. Supposing that the different chromosomes have different physiological values, or represent different hereditary characters, as maintained by Boveri ('02), we find in the behavior of the chromosomes in the germ cells of the Aphid exactly the conditions required by Mendel's Law of Heredity. The characters represented by the 5 constantly different chromosomes would be segregated at each recurrence of sexual reproduction, giving germ cells pure with respect to each of the 5 different characters or sets of correlated characters represented by the five chromosomes. During the whole series of parthenogenetic generations the same paternal and maternal series of chromosomes is maintained by longitudinal division, there is no amphimixis and no apparent chance for variation unless it be a change in dominance of certain characters, due to external conditions; for example, the winged-character and the sex-character.

The constant recurrence of this single or double series of chromosomes of the same relative form and size, is one point more in support of the hypothesis of the individuality of the chromosomes, strongly advocated by Rabl ('85) and Boveri ('87, '88, '91, '02). Recent papers by Sutton ('02) on Brachystola and Baumgartner ('04) on Gryllus show similar form and size relations of chromosomes; but in the Aphid one has the advantage of working with a smaller number, where each individual chromosome can be distinguished from all others of the series appearing in a winter egg or a spermatocyte.

2. *Maturation of Parthenogenetic Eggs.*

In comparing the results of various authors on this subject, one meets with great variations in different parthenogenetic forms. In the drone bee (Blochmann, '88–'89) two polar bodies are found, and Petrunkewitsch ('01) states that reduction occurs in the second maturation division, the normal number of chromosomes probably being restored by a subsequent longitudinal splitting of the chromosomes without mitosis. In Liparis dispar, Bombyx mori and Ocneria dispar (Platner, '88–'89, Henking, '92), two polar bodies are given off by the occasional parthenogenetic eggs and both sexes are produced. Weismann ('91), in attempting to bring these cases into line with his views of maturation and fertilization, says, "The nucleoplasm of certain eggs possesses a greater power of growth than that of a majority of the eggs of the same species, while in the case of the bee every ovum possesses a power of growth sufficient to double its nuclear substance." Petrunkewitsch's explanation of the presence of the normal number of chromosomes in somatic cells of the male bee sounds more like an echo of Weismann's argument than like the result of actual observation.

In Rhodites rosæ (Henking) there are two maturation divisions but no reduction of chromosomes in eggs that produce females. Males are rare and the maturation of the male egg is not known. In Artemia salina (Brauer, '94) either one polar body only is formed, or a second division occurs and the resulting nucleus conjugates with the egg nucleus. Here again the female and male generations seem not to have been distinguished.

Weismann and Ischikawa ('88) found only one polar body in the parthenogenetic eggs of several rotifers and crustaceans, no statement being made in regard to the male generations. Mrazek ('97), and Erlanger and Lauterborn ('97) found that in Asplanchna, a rotifer, the parthenogenetic female eggs gave off one polar body, while the parthenogentic male eggs formed two, and there was no indication of a union of the second polar body with the egg nucleus.

In Hydatina senta (Lenssen, '98) the first maturation division in parthenogenetic female eggs goes only as far as the metaphase; there is no reduction, and the chromosomes (10 or 12) fuse to form the egg nucleus. In the male egg reduction occurs, 5 or 6 chromosomes appearing in the polar plates of the spindle. In Hydatina it is supposed that the first maturation division is suppressed in both the parthenogenetic and the sexual eggs. In the Aphid only one polar body is given off in the parthenogenetic egg—male or female—and there is no evidence of reduction in either male or female parthenogenetic egg.

Thus we find a series, beginning with forms where parthenogenesis is either occasional or continues for only one generation, when maturation appears to follow the usual course for fertilized eggs; and ending with Hydatina senta where the whole process is practically suppressed in the parthenogenetic female eggs, and the Aphid where one maturation division without reduction remains in both male and female parthenogenetic eggs. We are thus led to question the importance of the second polar body in determining the male sex, also to question the view that parthenogenesis is due to the suppression of one or both maturation divisions, and to suggest that the various degrees of suppression of maturation phenomena in parthenogenetic eggs may be a more or less simple and perfect adaptation to a necessity of continued parthenogenetic reproduction, *i. e.*, the retention of the full double series of maternal and paternal chromosomes throughout the parthenogenetic portion of the cycle. The whole subject needs further investigation from the standpoint of the determination of sex.

3. Determination of Sex.

As the male sex cells of the Aphid contain no "accessory chromosome," McClung's theory of sex-determination need not be discussed in this connection. The question naturally arises whether the "accessory chromosome" is to be found in any of the parthenogenetic insects or crustaceans.

Castle ('03) in his recent paper on "The Heredity of Sex" attempts to place the sex-character in the same category with other hereditary chracters and to apply to it the principles of Mendel's Law of Heredity—dominance and segregation. He says on page 198, "A study of sex-heredity in parthenogenetic animals shows (1) that in such animals the female character uniformly dominates over the male whenever the two are present together," and on page 199, "With a single exception, we know that in uninterrupted parthenogenetic reproduction, as it occurs in the Daphnidæ and Rotiferæ at certain seasons, the parthenogenetic egg forms only one polar cell, and the animal developing from such an egg is invariably female, or more correctly ♂ (♀), the male character being recessive," and further on, "At the return to sexual reproduction, the parthenogenetic mother produces eggs which form a second polar cell, and from such eggs only males develop. It is clear, then, that in the second maturation division the female character has been eliminated from the egg, for were it still there, it must from its nature dominate."

As an exception Castle cites Rhodites rosæ, in which according to Henking the parthenogenetic eggs produce two polar bodies, but no reduction occurs, and therefore no segregation of sex characters. Castle assumes that the occasional egg which produces a male, does, in some way, eliminate the female character. Hydatina senta is also cited: according to Lenssen ('98) no polar body is formed and there is no reduction in the female egg, while in the male egg one maturation division occurs with reduction. Castle supposes in the latter case that the first maturation division is regularly suppressed as Sobotta ('99) has found to be the case in the mouse.

In the Aphid only one polar body is formed in the female and also in the male parthenogenetic egg, and there is no reduction of chromosomes in either case. If the sex character resides in one of the chromosomes, it is certainly not eliminated from the male egg. How shall we harmonize the conditions in the Aphid with Castle's theory? We cannot argue as he does for Bombyx mori, Ocneria dispar and all normally diœcious animals that there is no uniform dominance of one sex over the other. How then shall we account for the appearance of males, if all parthenogenetic Aphids are sex-hybrids with the female character usually dominant? The only possible argument seems to be that certain favorable conditions (warmth and abundant food) determine that the female sex-character shall continue dominant, the male sex-character recessive; while certain other conditions (not yet definitely determined) cause the male sex-character to become dominant and the female character recessive. A similar change in dominance may be imagined to account for the presence or absence of wings in both the parthenogenetic and the sexual generations. According to this theory we must suppose all of the oögonia to be alike in their hereditary characters—all sex-hybrids as well as hybrids in respect to other maternal and paternal characters. In addition, we must suppose that in parthenogenetic eggs, which undergo no reduction, dominance of certain characters can be reversed by external circumstances. This may of course occur at a very early stage in the history of the germ-cells, making the eggs at the time of maturation virtually male or female.

The case cited of two Aphids, each containing both parthenogenetic and winter ovaries (Fig. 20), and also showing that a parthenogenetic ovary may, after giving off parthenogenetic eggs, change to a winter ovary, the sexual form, is strong evidence that the ovarian oöcytes of the Aphid may be affected by external conditions. Whether the degeneration of certain oöcytes in all ovaries which are to produce winter eggs, can be considered evidence that there are two kinds of eggs, those that produce parthenogenetic young and those that require fertilization in order that they may develop, is not clear.

Beard ('02) says, in discussing parthenogenesis, "When in a parthenogenetic form, a long series of one sex appears, the eggs of the other sex must have been either delayed or suppressed." According to Beard's theory we must suppose that in the Aphid there are both male and female eggs, or at least such germ-cells, produced, and that all the male eggs are suppressed in the female generations while all the female eggs are suppressed in the male generations. There is no histological evidence of any such degeneration of the germ-cells.

In trying to fit the Mendelian theory of dominance, as elaborated by Castle, to the sex-conditions in Aphids, we meet with a peculiar contradiction in the fact that the same external conditions lead to the production of males and of sexual females. On the Œnothera this condition is very conspicuous, for in the autumn parthenogenetic reproduction is completely changed over into the sexual method of reproduction. Certain apterous individuals are producing male offspring, and at the same time or slightly earlier other winged individuals are producing the winter egg-layers. Three generations at least are involved in the winter egg production—an apterous generation followed by a winged generation and that by the apterous sexual females. In the case of the males only two generations are necessarily involved, an apterous generation and the generation of winged males. The food conditions which probably lead to the change in method of reproduction, may therefore differ in degree, the earlier conditions starting the sexual female line, and later conditions the male line. In favor of this argument is the fact that on the rose a scattered generation of sexual females is often met with before there are any males and before the regular female sexual generation appears.

It is perfectly evident that histological study of the germ-cells combined with observation of the living insects has not settled the question of sex-determination in the Aphid, but it has, in a way, cleared the field for interpretation of the results of experiment. We know (1) that there is no "accessory chromosome," and (2) that the formation of a second polar body with reduction of chromosomes does not occur in the male generation.

In the discussion of sex-determination by Cuénot ('99), Beard ('02), O. Schultze ('02), and Lenhossek ('03) we find that the evidence is overwhelmingly on the side of the view that sex is determined in the egg; but to the question how sex is determined in the egg, no thoroughly convincing answer has yet been given.

V. SUMMARY OF RESULTS.

1. There is no reduction in the number of chromosomes in the female parthenogenetic egg.

2. The 10 chromosomes form 5 pairs of different form and size.

3. The polar body, at first extruded from the egg, later becomes sunken in the cytoplasm, but takes no part in the development of the embryo.

4. The early segmentations are nuclear only, cell walls coming in at a later stage.

5. The young embryo takes in yolk material (the secundäre Dotter) from the vitellaria, through a definite valvular opening in the oviduct.

6. In the maturation of male eggs only one polar body is given off, and there is no reduction in the number of chromosomes.

7. The offspring of one parthenogenetic female are either all females or all males.

8. The ovaries of the parthenogenetic and of the sexual females may be originally identical in structure, as shown (1) by the presence of both kinds of ovaries in the same individuals; (2) by the degeneration of about half of the oöcytes in the posterior half of the ovary of the sexual female embryo. The significance of the latter phenomenon is uncertain.

9. The reduced number of chromosomes appears in the polar spindles of the winter eggs, and both divisions are longitudinal.

10. The somatic number of chromosomes appears in the spermatogonia.

11. Reduction in the spermatocytes is effected by longitudinal pairing of the chromosomes immediately before the first maturation mitosis.

12. The first maturation division probably separates paired chromosomes and the second is a longitudinal division of the original univalent chromosomes.

13. The delay in separation of one pair of chromosomes in the first maturation division is probably due to a different linin connection from the others, rather than to any physiological peculiarity.

14. There is no "accessory chromosome" in the male germ-cells of aphids.

Bryn Mawr College,
Dec. 20, 1904.

LITERATURE.

Balbiani, E. G., '69–'72.—Mémoire sur la génération des aphides. Ann. Sc. Nat. Ser. 5, Zool. T. 11, 1869; T. 14, 1870; T. 15, 1872.

Baumgartner, W. J., '04.—Some new Evidence for the Individuality of the Chromosomes. Biol. Bull. vol. viii, No. 1.

Beard, J., '02.—The Determination of Sex in Animal Development. Zool. Jahrb. vol. xvi.

Blochmann, F., '87.—Ueber die Richtungskörper bei Insekteneiern. Morph. Jahrb. vol. xii.

'88.—Ueber die Richtungskörper bei unbefruchtet sich entwickelnden Insekteneiern. Verh. naturh. med. Ver. Heidelberg, N. F., vol. iv, No. 2.

Bonnet, Ch., '45.—Traité d'Insectologie ou observations sur quelques espèces de Vers d'eau douce et sur les Pucerons. Paris. Durand.

Boveri, Th., '87.—Ueber Differenzierung der Zellkerne während der Furchung des Eies von Ascaris meg. Anat. Anz., vol. ii.

'88.—Zellenstudien II. Jena Zeitschr., vol. xxii.

'91.—Befruchtung. Merkel u. Bonnet's Ergebnisse, vol. i.

'02.—Ueber mehrpolige Mitosen als Mittel zur Analyse des Zellkerns. Verh. d. phys.-med. Ges. Würzburg. N. F., Bd. xxxv.

Brauer, A., '94.—Zur Kenntniss der Reifung des parthenogenetisch sich entwickelnden Eies von Artemia salina. Arch. mikr. Anat., vol. xi.

Castle, W. E., '03.—The Heredity of Sex. Bull. of the Mus. of Comp. Zoöl. Harvard College, vol. xl, No. 4.

Cuénot, L., '99.—Sur la détermination du sexe chez les animaux. Bull. Sci. Fr. Belgique, vol. xxxii.

Erlanger u. Lauterborn, '97.—Ueber die ersten Entwickelungsvorgänge im parthenogenetischen und befruchteten Räderthierei. Zool. Anz., vol. xx.

Henking, H., '92.—Untersuchungen über die ersten Entwickelsvorgänge in den Eiern der Insekten. Zeit. wiss. Zool., vol. liv.

Kyber, J. F., '15.—Einige Erfahrungen und Bemerkungen über Blattläuse. Germar's Magazin der Entomologie, t. 1–2c.

Lenhossek, M. v., '03.—Das Problem der geschlechtsbestimmenden Ursachen. Jena. Fischer.

Lenssen, J., '98.—Contribution à l'étude du dévelopment et de la maturation des oeufs chez Hydatina senta. La Cellule, vol. xiv.

Leydig, T., '50.—Einige Bemerkungen über die Entwickelung der Blattläuse Zeit. wiss. Zool., vol. ii.

McClung, C. E., '02.—The Accessory Chromosome—Sex-Determinant? Biol. Bull., vol. vii, Nos. 1 and 2.

Mrazek, Al., '97.—Zur Embryonalentwickelung des Gattung Asplanchna. Jahresb. böhna Ges., vol. ii.

Petrunkewitsch, A., '01.—Die Richtungskörper und ihr Schicksal im befruchteten und unbefruchteten Bienenei. Zool. Jahrb., vol. xiv.

Platner, G., '88.—Die erste Entwickelung befruchteter und parthenogenetischer Eier von Liparis dispar. Biol. Centralbl., vol. viii, No. 17.

'89.—Ueber die Bedeutung der Richtungskörperchen. Biol. Centralbl., vol. 8.

Rabl, C., '85.—Ueber Zellteilung. Morph. Jahrb., vol. x.

Schultze, O., '02.—Was lehren uns Beobachtung und Experiment über die Ursache männliche und weibliche Geschlechtsbildung bei Tieren und Pflanzen? Sitz. ber. der phys.-med. Ges. Würzburg. 1902.

Sobotta, J., '99.—Ueber die Bedeutung der mitotischen Figuren in den Eierstockeeiern der Säugetiere. Festschr. phys.-med. Ges. Würzburg. 1889.

Sutton, W. S., '02.—On the Morphology of the Chromosome Group in Brachystola magna. Biol. Bulletin, vol. iv, No. 1.

Weismann, A., '63.—Die Entwickelung der Diptera im Ei. Zeit. wiss. Zool., vol. xiii.

'92.—Essays on Heredity. Vol. ii. Oxford. 1892, p. 174.

Weismann and Ischikawa, '88.—Weitere Untersuchungen zum Zahlengesetz der Richtungskörper. Zool. Jahrb., vol. iii.

Will, L., '83.—Zur Bildung des Eies und Blastoderms bei der viviparen Aphiden. Arb. Zool. Zoot. Inst. Würzburg. Vol. vi.

'88.—Zur Entwickelungsgeschichte der viviparen Aphiden. Biol. Centralbl., vol. viii, No. 5.

'89.—Entwickelungsgeschichte der viviparen Aphiden. Zool. Jahrb., vol. iii.

Witlaczil, Em., '84.—Entwickelungsgeschichte der Aphiden. Zeit. wiss. Zool., vol. xl.

DESCRIPTION OF PLATES.

With the exception of Figs. 20 and 21, the figures are all camera drawings, made with Zeiss obj. 2 mm., oc. 8, except Figs. 1, 14, 15 and 19, which were drawn with Zeiss obj. 2 mm., oc. 4, and Fig. 22, for which Zeiss obj. D, oc. 4 was used. Plates II and III have been reduced one-tenth.

PLATE I.

Fig. 1. Parthenogenetic ovary from an embryo of Aphis rosæ. *a* = a developing ovum. *b* = stalk of the next ovum in the egg-string.

Fig. 2. Oöcyte from above ovary.

Fig. 3. Oöcytes at the beginning of their growth period, showing chromosomes but no nucleolus.

Figs. 4–6. Later growth stages of the oöcyte.

Fig. 7. Equatorial plate of the polar spindle, showing 10 chromosomes of 5 different sizes and shapes *a–e*.

Fig. 8. Polar spindle in anaphase. Polar body already separated from the egg.

Fig. 9. A later stage, showing the polar body (*p*), and the egg-nucleus (*n*).

Fig. 10. An egg containing the first segmentation spindle, and the polar body (*p*) somewhat sunken in the cytoplasm of the egg.

Fig. 11. Segmentation stage with resting nuclei and polar body (*p*) lying in a vacuole in the cytoplasm of the unsegmented egg.

Fig. 12. Segmentation stage, showing polar body (*p*) and mitotic figures. The chromosomes of the equatorial plate are lettered *a–e* to correspond to those of the equatorial plate of the polar spindle in Fig. 7.

PLATE II.

Fig. 13. Later segmentation stage, showing polar body (*p*) probably degenerating.

Fig. 14. Young parthenogenetic embryo just beginning to take in yolk (*a*) through a valvular opening in the oviduct (*b*).

Fig. 15. A slightly later stage of the same process.

Fig. 16. Resting oöcyte from an ovary of Aphis œnotheræ, all the embryos male.

Fig. 17. Section of a segmenting egg from an individual containing male embryos, showing one polar body (*p*).

Fig. 18. Part of a section similar to the above, showing chromosomes not so completely fused.

Fig. 19. Ovary from a female embryo of the sexual generation, showing degenerating oöcytes (*a*).

Fig. 20. Freehand sketch of a mixed group of ovaries, consisting of five winter ovaries (*a*) with winter eggs (*b*), and one parthenogenetic ovary (*c*) with developing eggs and embryos (*e*).

Fig. 21. Freehand sketch of egg-string from the other side of the same individual, showing two parthenogenetic embryos (*e*), a winter egg (*b*) and a winter ovary (*a*).

PLATE III.

Fig. 22. Oblique section of a winter egg, showing entrance of secondary yolk from without. *a* = yolk cell (nucleus in next section). *b* and *c* = fragments of nuclei.

Fig. 23. Equatorial plate of first polar spindle in winter egg of Aphis rosæ. The five chromosomes are lettered *a–e* corresponding to the five pairs in Figs. 7 and 12.

Fig. 24. First polar spindle, metaphase. The stippled chromosome is a part of one which appears in *a*.

Fig. 25. First polar body (p^1) containing five chromosomes, and second polar spindle. Two chromosomes appear in two sections *b* and *c*, and a part of one belonging to the first polar body is seen in section *a*.

Fig. 26. First polar body (p^1); chromosomes of second polar body (p^2); and female pronucleus forming (*n*).

Fig. 27. Male and female pronuclei. The arrows indicate the path of the sperm nucleus.

PLATE IV.

Fig. 28. Resting spermatogonium of Aphis œnothoræ.

Fig. 29. Spermatogonia preparing for mitosis, showing both nucleolus and chromosomes.

Fig. 30. Spermatogonium showing 9 of the 10 chromosomes, and no nucleolus.

Fig. 31. Equatorial plate of spermatogonial mitosis.

Fig. 32. Spermatocyte of first order in resting stage.

Fig. 33. Spermatocytes of first order, showing (*a*) eight of the ten chromosomes not paired and (*b*, *c*, *d*) chromosomes of the same size in pairs.

Fig. 34. Spermatocytes before division, possibly a "synapsis" stage.

Fig. 35. Equatorial plate of first maturation mitosis, showing variations in arrangement of chromosomes (*a*, *b*, *c*), and double chromosomes, (*c*) chromosomes lettered as in Figs. 7, 12 and 23.

Fig. 36. Side view and oblique view of equatorial plate of first maturation mitosis showing double chromosomes.

Fig. 37. Anaphase of first maturation mitosis showing "lagging" chromosome.

Fig. 38. Later stage of the same.

Fig. 39. A pair of spermatocytes of the second order in a partial resting stage, the lagging chromosomes still connected.

Fig. 40. Equatorial plate of second maturation mitosis, chromosomes lettered as in Figs. 7, 12, 23 and 35.

Fig. 41. Anaphase of second maturation mitosis.

Fig. 42. Spermatids in various stages.

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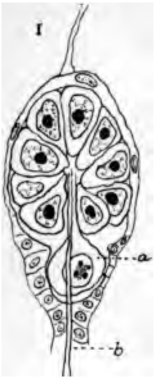

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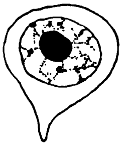

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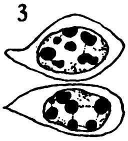

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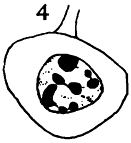

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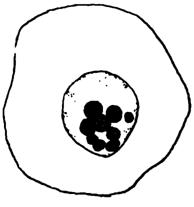

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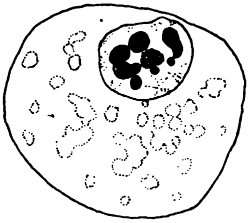

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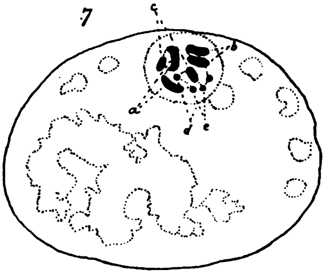

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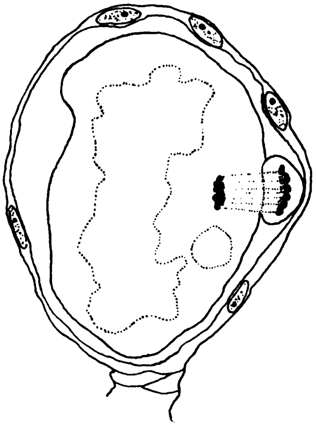

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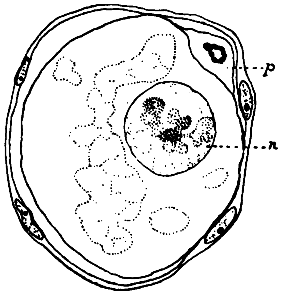

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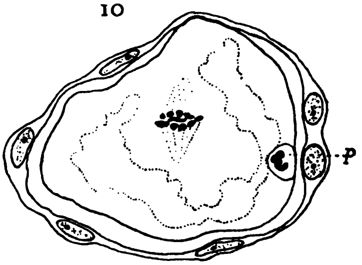

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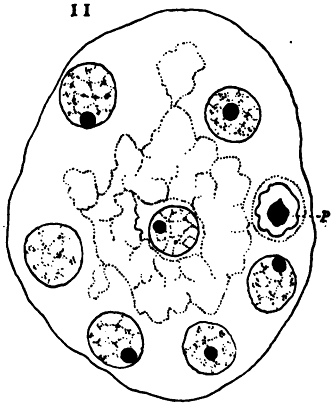

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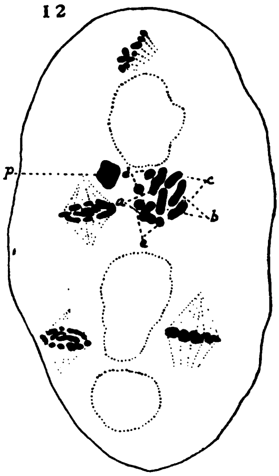

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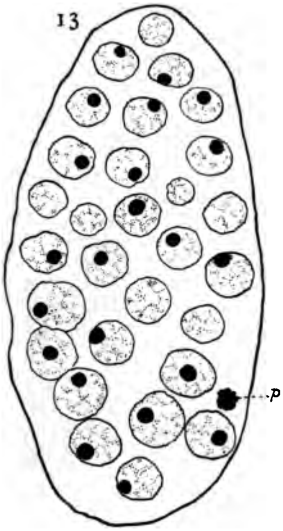

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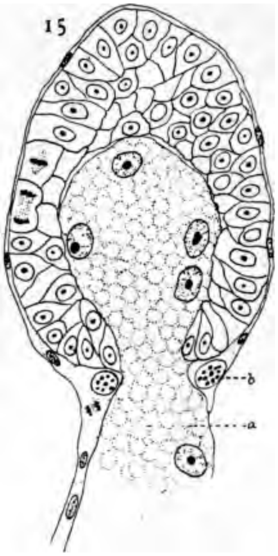

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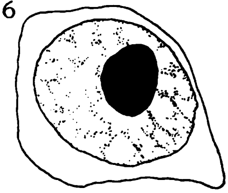

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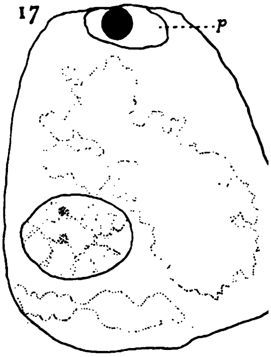

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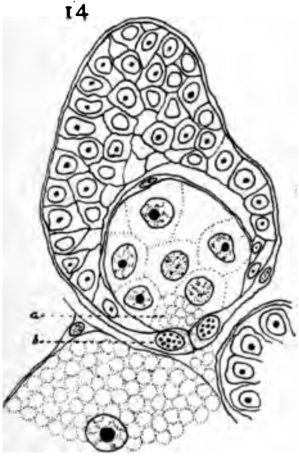

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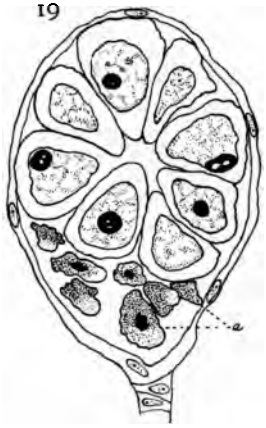

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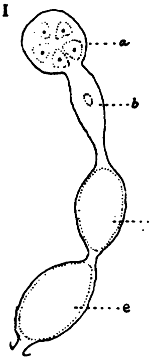

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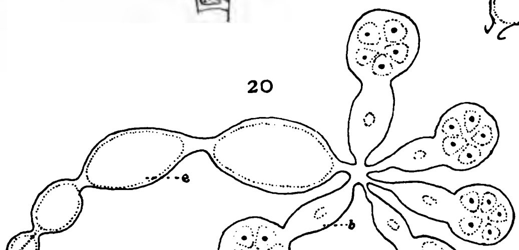

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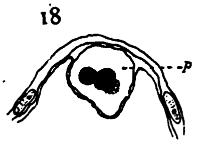

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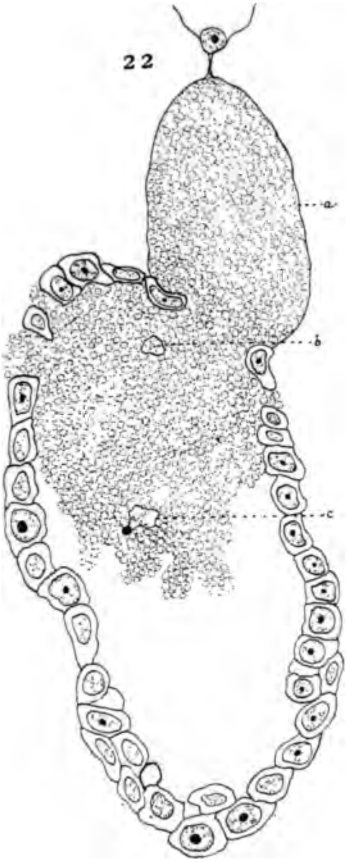

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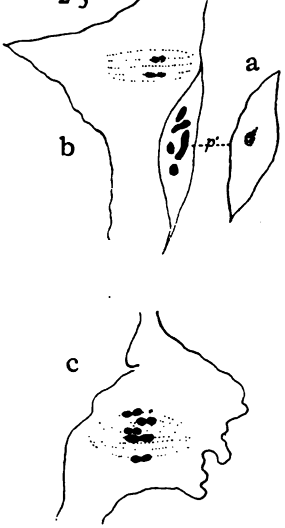

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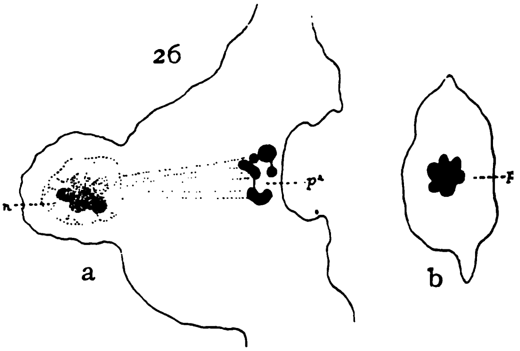

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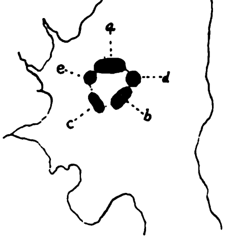

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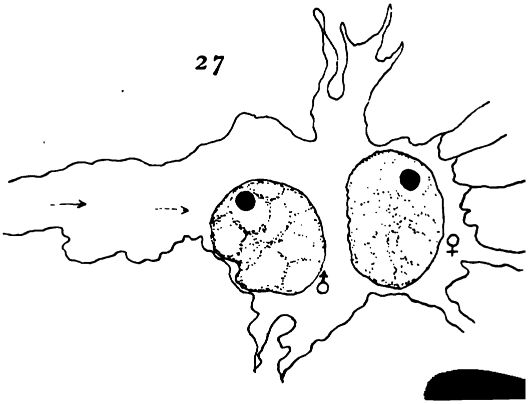

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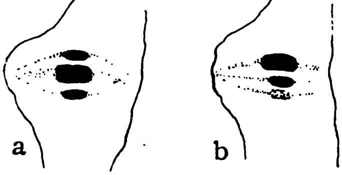

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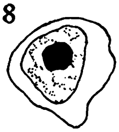

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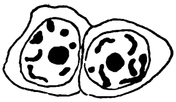

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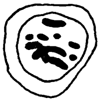

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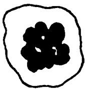

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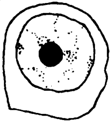

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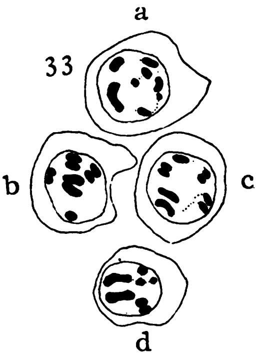

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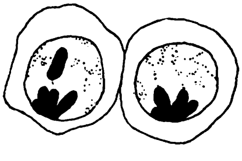

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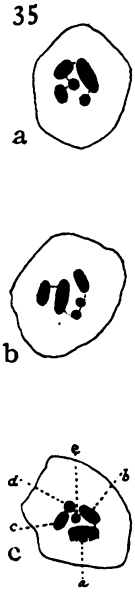

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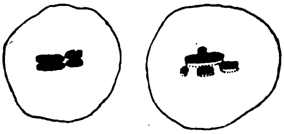

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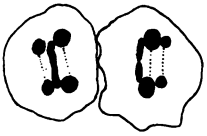

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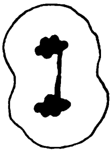

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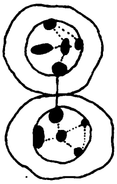

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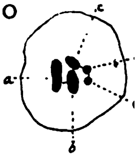

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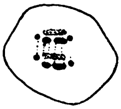

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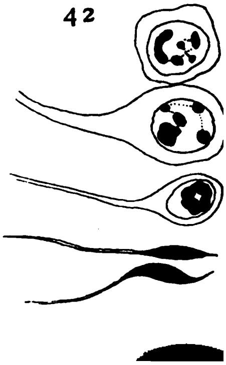


N. M. S. del.

REGENERATION IN POLYCHŒRUS CAUDATUS.

BY

N. M. STEVENS AND A. M. BORING.

PART I. OBSERVATIONS ON LIVING MATERIAL.

BY

N. M. STEVENS.

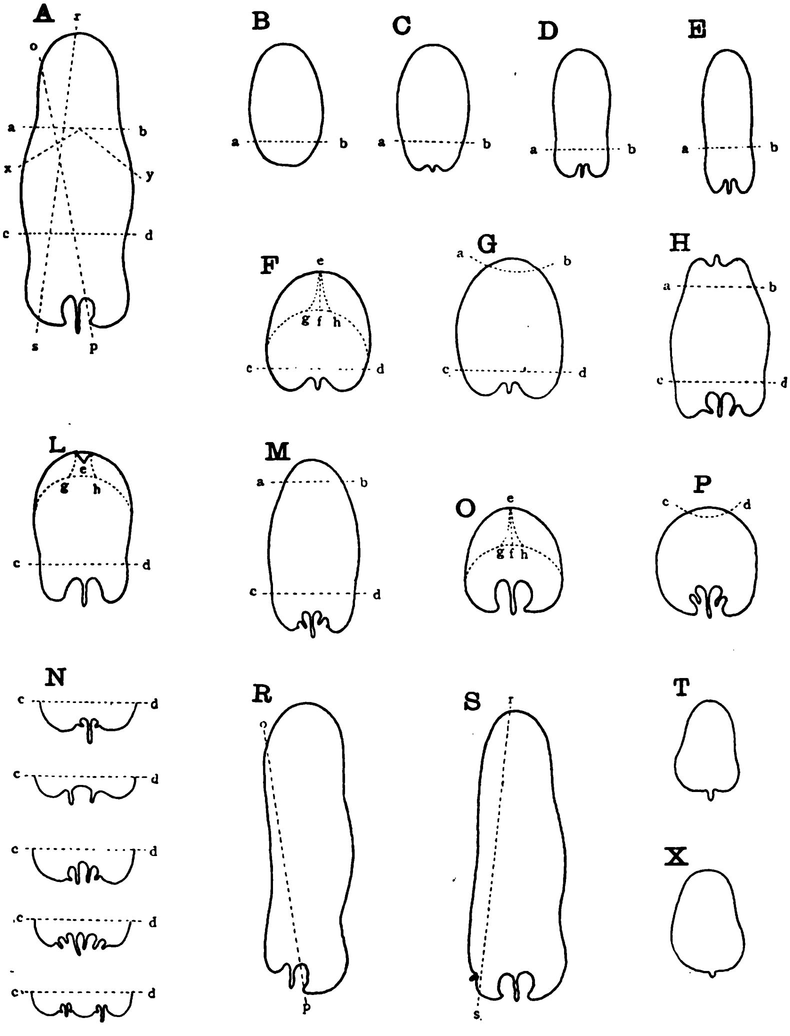
WITH 21 FIGURES.

While enjoying the hospitality of the Hopkins Seaside Laboratory at Pacific Grove, Cal., the past summer, I made a few experiments to test the powers of regeneration of the red acœlous flatworm, Polychœrus caudatus, which abounds there in shallow tide-pools on the underside of stones and shells and on Ulva. The object of the experiments was a comparison of the regeneration of this form which has no definitely differentiated organs—eyes, central nervous system, pharynx, etc.—with the more highly organized fresh-water Planarians, as well as with the results of Schultz ('02) and Child ('04) on Leptoplana and other marine forms which show very incomplete anterior regeneration.

Method.

In most of the experiments, the worms were cut into three nearly equal parts as in Fig. A, *a—b*, *c—d*. These parts will be spoken of as head-pieces, middle-pieces and tail-pieces. The material was kept in covered glass dishes, somewhat shaded, and the sea-water was changed morning and evening.

Regeneration in general was much slower than in fresh-water Planarians. The animals are very sluggish normally, and the pieces moved but little even when disturbed by changing the water, the head-pieces, however, being much more active than the middle-pieces and tail-pieces. The tail-pieces continued to deposit eggs for several days as freely as did the entire worms, and the eggs developed normally.



A.—Whole worm showing planes of section. B—D.—Head-pieces after 2 weeks' regeneration. E.—Head-piece after 4 weeks' regeneration. F.—Middle-piece after 2 weeks, showing ventral union of anterior edges (*e*—*f*), V of new tissue (*g*—*e*—*h*), and posterior regeneration. G.—Middle-piece after 2 weeks, showing anterior regeneration where the edges have not united as in F. H.—Middle-piece after 19 days, showing heteromorphic tail. L.—Middle-piece after 4 weeks, showing more advanced anterior regeneration of the type shown in F. M.—Middle-piece after 4 weeks, showing anterior regeneration of the type shown in G. N.—Posterior regeneration of middle-pieces, showing supernumerary appendages. O—P.—Regeneration in tail-pieces, 2 weeks. R—S.—Lateral regeneration, 4 weeks. T—X.—Young worms, still in jelly, with appendages just developing.

Head-pieces.

These pieces very soon began to produce new tissue at the cut surface as in other Planarians. Among the 40–50 pieces in a series, at the end of two weeks, the stages of posterior regeneration shown in Figs. B, C and D were found with all intermediate stages. A rounded mass of new tissue of considerable size forms posterior to the cut surface, *a—b*, before the characteristic notch and appendage appear. Continued regeneration adds to the length of the new part while the old part decreases in width and the whole piece gradually assumes the typical form. The notch, at first broad and shallow, becomes deeper and narrower, and the appendage longer. The new part assumes the characteristic pigmentation of the adult tail-region, and a digestive region forms anterior to the line of section, *a—b*. Regeneration of these pieces was not followed longer than four weeks, when most of the pieces had assumed the form shown in Fig. E, where, if one compares with Figs. A and B, morphallaxis is very apparent.

Middle-pieces.

In these pieces posterior regeneration proceeded somewhat differently. New tissue appeared along the whole of the cut surface, but was so distributed as to form a median notch from a very early stage. One or more appendages appeared earlier than in the regeneration of head-pieces. Figs. F and G show the usual amount of posterior regeneration after two weeks, and Figs. H, L, M and N after four weeks. In all of these pieces the notch is still much broader and more widely open than in the typical form A. The multiple appendages shown in the figures were at first thought to be a peculiarity connected with regeneration; but examination of many normal worms showed that, though one appendage is the typical structure, still all the variations observed in regeneration are to be found in normal adult worms. These variations are, however, far more frequent in regeneration, and more frequent in middle-pieces than in head-pieces, where, as a rule, only one appendage develops. These observations suggested a comparison with the formation of the tail-region in the embryo. Figs. T and X show two young worms, ten to twelve days after the eggs were laid, and still in the jelly which enveloped the eggs. The appendage has appeared but not the characteristic notch. Posterior regeneration in head-pieces (Figs. B and C) follows more nearly the embryonic method of tail development than does that of middle-pieces, where regeneration from the beginning seems to be based on the adult form of the tail-region which has been removed.

Anterior regeneration varied greatly in different lots of material and in different pieces of the same series. There are, however, two distinct types. In most cases the cut anterior end, *a—b*, folded together ventrally and the portions on either side of the median line united as shown in Fig. F, *e—f*. In the first set of pieces no anterior regeneration occurred while the material was under observation. In another set, in which all the pieces regenerated better, a few at the end of two weeks showed a V of new tissue between the united cut edges, Fig. F, *g—e—h*. At the end of four weeks such pieces had developed as in Fig. L, *g—e—h*, and later some of them produced typical worms. As the union of the cut edges, as in Fig. F, *e—f*, appeared to hinder regeneration in many cases, an attempt was made to prevent the union of the edges or to remove the hindrance later on. Pieces were cut as in Fig. A, *x—y*, or with a sharper angle, but the cut edges still curled under and united as before. Cutting the line of union was equally unsuccessful. There were a few pieces which contracted at the anterior end without folding under and uniting; these regenerated as shown in Figs. G and M, and in due time produced worms of typical form. Anterior regeneration was, however, in all cases less rapid than posterior. One piece produced a heteromorphic tail, Fig. H. This individual did not crawl normally, but half crawled, half swam with great difficulty on its back or side. This was the only case of heteromorphosis observed.

Tail-pieces.

Anterior regeneration of tail-pieces was of the two general types described for middle-pieces and illustrated in Figs. O and P. In general it was less rapid and less complete than in middle-pieces.

Lateral Regeneration.

A few worms were cut longitudinally in various ways. Regeneration occurred along the whole cut surface as in other forms, the new material being distributed in proportion to the amount removed. Figs. R and S show the result in two cases of diagonal section, as in Fig. A, *o—p*, and *r—s*, after four weeks. In Fig. R the posterior end on the regenerating side is in approximately the same condition as in cases of entire posterior regeneration. In Fig. S, an abnormal notch and appendage has developed near *s*, as though the notch and appendage were a necessary accompaniment of posterior regeneration without regard to the presence of the same structure in the old part. This phenomenon also recalls the supplementary heads and tails described by Morgan ('01) and others, as appearing on long obliquely or longitudinally cut surfaces.

General Discussion.

The results of the experiments show that in Polychœrus caudatus anterior regeneration at different levels may proceed much as in many fresh-water forms (Figs. G, M and P), or it may be prevented or delayed, not by muscular contraction and union of the muscle bands, as described by Schultz ('02), but by a folding under and union of the cut edges. (Fig. F, *e—f*.) That such union of the cut edges is not an insuperable hindrance to regeneration in this form is proved by such cases as are shown in Figs. F, L and O, where regeneration begins with the formation of a V of new tissue and ends with the production of a typical head-region.

In Polychœrus there is no axial gut (Bardeen, '01), nor is there a central nervous system to influence regeneration (Lillie, '00; Child, '04). The fact that head-pieces, which are more active, regenerate more rapidly than middle-pieces or tail-pieces, might be held to support Child's theory that "there is a close parallelism between the rapidity, amount and completeness of regeneration and the characteristic activity of the part concerned;" but the difference in rate of regeneration and morphallaxis is not proportionate to the difference in activity, for head-pieces are easily stimulated into activity by changing the water or jarring the dish, while middle-pieces and tail-pieces hardly move at all during the first two weeks unless violently disturbed. The difference in activity is great, while the difference in rate of regeneration is comparatively small.

So far as regeneration in Polychœrus has been tested by these experiments, it seems to be largely a question of "organization" and "totipotence" of material (Morgan, '04) modified in many cases by the folding under and uniting of the anterior cut surfaces.

It is the intention of the authors to supplement this work with further experiments during the coming summer.

PART II. HISTOLOGY.

BY

A. M. BORING.

WITH 2 PLATES AND 1 FIGURE IN THE TEXT.

After working on the external features of the regeneration of Polychœrus caudatus in California during the past summer, Miss Stevens brought back to Bryn Mawr some preserved material—the whole flatworms and pieces that had regenerated for varying lengths of time. The simplicity of structure and the lack of any great differentiation of tissue, made it a matter of interest to work out the histological side of the regeneration of this form, in order to see whether it differs in any essential points from the method of regeneration in more highly differentiated forms, such as Planaria simplicissima, described by Stevens ('01), and Planaria maculata, worked out by Curtis ('02) and Thacher ('02).

Technique.

The material had been fixed in a mixture of corrosive sublimate and acetic acid, the regenerating pieces at the end of one, two, five, seven, ten, fourteen, and twenty-eight days. After being hardened in the alcohols, and embedded in paraffine, the whole worms were sectioned in transverse and sagittal planes, and the regenerated pieces in transverse, sagittal, and frontal planes. The sections were stained in Delafield's hæmatoxylin, followed by orange G. This combination gives a good differentiation of the various tissues. The reproductive cells stain purple, the mucus blue, the nuclei of the parenchyma cells brown, the parenchyma itself pale yellow, the muscle cells deeper yellow, and the cilia usually form a slightly stained border at the margin of the sections, in parts of which the separate cilia can be distinguished.

Normal Structure.

Before describing the process of regeneration, it seems necessary to describe the normal structure, as this differs essentially from that of other Planarians, and has not been described in detail.

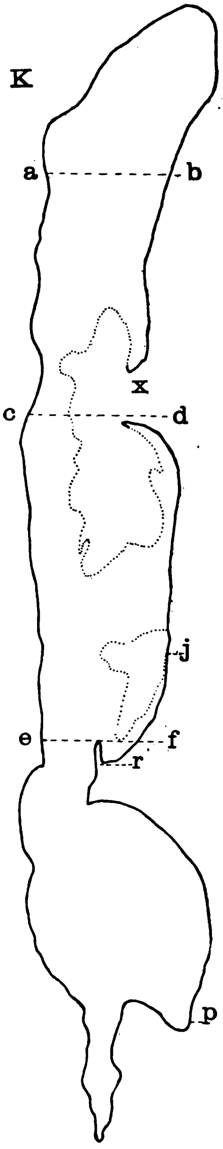


Fig. K is a sagittal section of a whole worm showing the general outline of the form and the location of the different openings; *x* is the digestive opening, *r* the female reproductive opening, and *p* the penis. It also shows the position of the cells that secrete the jelly in which the eggs are laid *j*.

Fig. 1 is a transverse section taken near the anterior end of the worm (Fig. K, *a*—*b*), showing the testis cells *t*, maturing spermatozoa *s*, mucus *m*, the parenchyma nuclei *n*, and the cilia *c*. Fig. 2 is a transverse section through the middle-region (Fig. K, *c*—*d*), showing in addition, egg cells *o*, the irregular digestive region *d*, containing some food *f*, and the digestive opening *x*. Fig. 3 is a transverse section near the posterior end (Fig. K, *e*—*f*), showing besides the foregoing features, the female reproductive opening *r*, and the jelly gland *j*. In these three sections, certain distinctions between the dorsal and ventral sides can be seen. Most of the mucus lies on the dorsal side. There are more nuclei on the ventral side than on the dorsal, and there is a marked aggregation of nuclei at the lateral edges of the ventral side. By comparing Fig. 4, a piece of the dorsal margin of a transverse section (similar to Fig. 2), with Fig. 5, a piece of the ventral margin, an additional difference appears, that of the arrangement of the muscle fibers. On the dorsal side, they are more regularly arranged, forming an outer circular and an inner longitudinal layer, while on the ventral side, there are no distinct layers. The apparent difference in the length of the cilia in these two figures (4 and 5) may be due to their being matted together in fixation.

There is no definite ectoderm or endoderm. The cells composing the mass of the body are the parenchyma cells, irregularly spindle-shaped, with large nuclei. (Fig. 4, *n.*) In many places, the outlines of these cells are so indefinite that it appears as though they merged into one another, forming a syncytium studded here and there with nuclei. Among these parenchyma cells are mucous cells, which have similar nuclei, but contain masses of a blue-staining secretion. (Fig. 4, *m.*) On the outer edge, where one would expect to find a definite ectoderm, these parenchyma cells are ciliated (Fig. 4, *c*), and stain a little more deeply, perhaps due to a cuticular secretion; but in no other way is the outer layer of cells different from the cells making up the mass of the body. This outer layer is not even arranged regularly, for the nuclei are at varying distances from the base of the cilia, and at irregular distances apart. The cells of the digestive region (Fig. 2, *d*)—it is not definite enough to be called a digestive tract—do not differ in any respect from the other parenchyma cells. In places pieces of crustaceans, which have been taken in as food, are found in between the cells near the digestive region (Fig. 2, *q*), showing that this cavity is continuous with the spaces between the loose parenchyma cells. At the opening of the digestive region (Fig. 2, *x*), a few of the cells are sometimes ciliated (Fig. 2, *c*) like the ectodermal parenchyma cells.

Muscle fibers are scattered throughout the parenchyma, but are accumulated especially among the ectodermal parenchyma cells (Fig. 4, *g*), around the female reproductive opening, and in the penis, of which they are the chief constituent. They vary much in size, in fact, so much that in sections stained with iron hæmatoxylin and orange G, some take the black and some the yellow color.

The reproductive cells are more distinctly differentiated than the other cells in these flatworms. They are not grouped into ovaries or testes, but they lie in definite positions among the parenchyma cells, and are discharged through definite openings, guarded by muscle cells having a sphincter-like arrangement. The testis cells (Fig. 2, *t*) extend along the lateral edge from near the anterior end to the penis which is an external muscular organ. (Fig.

K, *p*). The egg cells (Fig. 2, *o*) lie on each side of the median ventral line, extending from the region of the digestive opening back to the female reproductive pore. Just in front of this pore lie the cells which secrete the jelly in which the eggs are laid. (Fig. 3, *j*.)

This form has no central nervous system, no eyes or other sense organs, and no excretory system.

Regeneration.

The regeneration of this form is as simple as its structure. The worms were cut into three pieces as stated in Part I, a head-piece, a middle-piece, and a tail-piece. In the regeneration of Planaria simplicissima and of Planaria maculata, the old ectoderm stretches over the cut surface in a thin layer, but the regenerative process in Polychœrus caudatus is more like the regeneration after natural fission in Planaria maculata, as described by Curtis ('02), where the exposed surface simply heals over and embryonic cells migrate to that region and form the new tissue. In Polychœrus, the cells at the cut end secrete a cuticular substance and develop cilia. Sections of most of the pieces fixed two days after being cut, show short cilia at the cut end (Fig. 6, c_1) and the cells stain a little more deeply at the base of the cilia. In the five day sections, the cilia have reached their normal length. (Fig. 7, *c*.) By this time there is also a decided accumulation of nuclei at the regenerating end. Fig. 7 shows this, and a comparison of Fig. 7 with Fig. 6 clearly shows the progress of regeneration. This accumulation is not due to cell division, either in the regenerating end, or the old part. Cell division has been carefully looked for throughout the work, and the one or two cases which might possibly be interpreted as prophases of mitosis lose all significance from their rarity and the entire absence of actual mitoses; neither has any evidence of amitosis been discovered. Many of the nuclei in Fig. 7 have their long axes pointed toward the end, and the cells, as far as their outline can be made out, point in the same direction, indicating a streaming of parenchyma cells toward the regenerating region. In the whole worm, the parenchyma nuclei are accumulated on the ventral side and especially toward the lateral edge. In Fig. 9, a sagittal section some distance lateral to the median line, the accumulation of nuclei on the ventral side is continuous with the accumulation at the regenerating end *n*, suggesting this accumulation as the chief source of the cells in the new part. Some of the cells come from the dorsal side, but the evidence from the examination of many sections is convincing that the majority come from the ventral side.

The muscle cells must develop from the parenchyma cells *in situ*, as they appear below the ectodermal parenchyma only in pieces which have been regenerating several days. (Fig. 8, *g*.) In Fig. 6, a section of a piece before the accumulation of nuclei had begun, some fibers appear scattered irregularly through the parenchyma near the end, but these are probably old fibers, as this section shows no definite layer of muscle fibers below the ectodermal parenchyma at the regenerating end.

The seven-day and ten-day sections show an increase in the length of the new part, but no other new points. In two weeks, most of the new tissue has taken on the loose parenchymatous character of the old part, as shown by the spaces in the tissue and the more scattered position of the nuclei in Fig. 10, only the extreme end of the regenerated tissue still having the nuclei in close proximity and the cells densely packed together. (The dotted lines in Figs. 9–12 show approximately the boundary between old and new parts.)

In the regeneration of one of the oldest head-pieces, a new digestive opening has formed. (Fig. 11, *x*.) It is in all respects like the opening in a full sized worm, being situated about halfway between the anterior and posterior ends, and opening directly from the digestive region to the exterior. Middle-pieces of this age have the old digestive opening, but some distance posterior to this, at the base of the new tissue, there is an accumulation of parenchyma and muscle cells, as in Fig. 12, *j*, which can be recognized as the anlage of the penis, for the sperm has moved down near to this anlage. Anterior to the penis is a slight indentation *r* which may indicate the anlage of the female genital pore.

Sections show that anterior regeneration is always slower than posterior; there is less new tissue at the anterior end than at the posterior, and it keeps its compact character and accumulation of nuclei longer than the posterior, as shown by comparing Fig. 7, an anterior end, with Fig. 8, a posterior end of the same age. In some pieces in which the anterior end folded under to form a pocket, as described in Part I, no regeneration can be seen in the oldest stages, but in a few of these, the growth of new material between the united edges can be seen in section. (Fig. 13, *v.*) By studying the whole series of sections, this region can be identified as the place where the cut edges united. The accumulation of nuclei shows new tissue to be regenerating on both sides of the line of union.

A few cases of lateral regeneration were studied, but the sections showed no divergence from anterior and posterior regeneration.

The regeneration of Polychœrus caudatus is an excellent example of the remolding of the old tissue in a piece of an organism, into the tissues and form of the whole organism, without the assistance of cell division by mitosis or amitosis. This is what Morgan calls morphallaxis. Other flatworms in which regeneration has been worked out histologically, Planaria simplicissima and Planaria maculata, show a proliferation of new cells at the cut end, as well as the changes of form due to morphallaxis, but in Polychœrus the new part is formed wholly of cells which migrate from the old part. Regeneration in this form is, therefore, an example of morphallaxis, pure and simple.

Bryn Mawr College, Pa.
April 19, 1905.

LITERATURE.

BARDEEN, C. R., '01.—On the Physiology of Planaria maculata with Especial Reference to the Phenomena of Regeneration. Am. Journ. of Physiology, vol. v, 1901.

CHILD, C. M., '04a.—Studies on Regulation. IV. Some Experimental Modifications of Form Regulation in Leptoplana. The Journal of Exp. Zoölogy, vol. 1, No. 1, 1904.

'04b.—Studies in Regulation. V. The Relation between the Central Nervous System and Regeneration in Leptoplana: Posterior Regeneration. *Ibid.*, No. 3, 1904.

'04c.—Studies in Regulation. VI. The Relation between the Central Nervous System and Regulation in Leptoplana: Anterior and Lateral Regeneration. *Ibid.*, No. 4, 1904.

CURTIS, W. C., '02.—Life History, Normal Fission, and Reproductive Organs of Planaria maculata. Proc. Boston Soc. of Nat. Hist., vol. xxx, No. 7, 1902.

LILLIE, F. R., '01.—Notes on Regeneration and Regulation in Planarians. Am. Journal of Physiology, vol. vi, 1901.

MORGAN, T. H., '01.—Regeneration. Columbia Univ. Biol. Series, No. 7. New York. 1901.

'04.—An Analysis of the Phenomena of Organic 'Polarity.' Science, N. S., vol. xx, No. 518.

STEVENS, N. M., '01.—Notes on Regeneration Planaria lugubris (simplicissima). Archiv. für Entw., Bd. xiii, H. 3, 1901.

THACHER, H. F., '02.—Regeneration of a Pharynx in Planaria maculata. Am. Naturalist, vol. xxxvi, No. 428, 1902.

EXPLANATION OF PLATES.

Figs. 1, 2, 3, 9, 10, 11, 12, 13 were drawn with Leitz oc. 2, obj. 3, camera lucida.

Figs. 4, 5, 6, 7, 8 were drawn with Leitz oc. 2, obj. 1-12, camera lucida.

Figs. 1 to 8 are reduced one-half.

The following lettering is used in all the figures: *c*, cilia; c_1, cilia half developed; *d*, digestive region; *f*, food in digestive region; *g*, muscle fibers; *j*, jelly gland; *m*, mucus; *n*, nuclei of parenchyma cells; *o*, ova; *p*, penis; *q*, food among parenchyma cells; *r*, female reproductive opening; *s*, sperm; *t*, testis cells; *v*, new material; *x*, opening to digestive region; *y*, appendage.

PLATE I.

Fig. 1. Transverse section of whole worm near anterior end. (Fig. K, *a—b*.)

Fig. 2. Transverse section of whole worm near middle, through the digestive opening. (Fig. K, *c—d*.)

Fig. 3. Transverse section of whole worm toward posterior end, through female genital pore. (Fig. K, *e—f*.)

Fig. 4. Portion of dorsal margin of transverse section.

Fig. 5. Portion of ventral margin of transverse section.

Fig. 6. Sagittal section of anterior end after 2 days' regeneration, showing developing cilia.

Fig. 7. Sagittal section of anterior end after 5 days' regeneration, showing accumulation of nuclei.

Fig. 8. Sagittal section of posterior end after 5 days' regeneration, showing appearance of muscle fibers.

PLATE II.

Fig. 9. Lateral sagittal section of middle-piece after 5 days' regeneration, showing accumulation of nuclei, *n*.

Fig. 10. Median sagittal section of middle-piece after 2 weeks' regeneration, showing tissue with the loose character of the old. (Exceptionally rapid regeneration.)

Fig. 11. Sagittal section of head-piece after 4 weeks' regeneration, showing the new digestive opening.

Fig. 12. Sagittal section of middle-piece after 4 weeks' regeneration, showing anlage of penis and of female genital pore.

Fig. 13. Transverse section of middle-piece (4 weeks) with a "pocket," showing triangle of new material, *v*.

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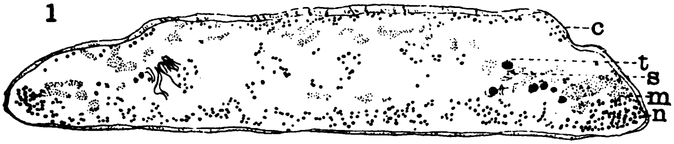

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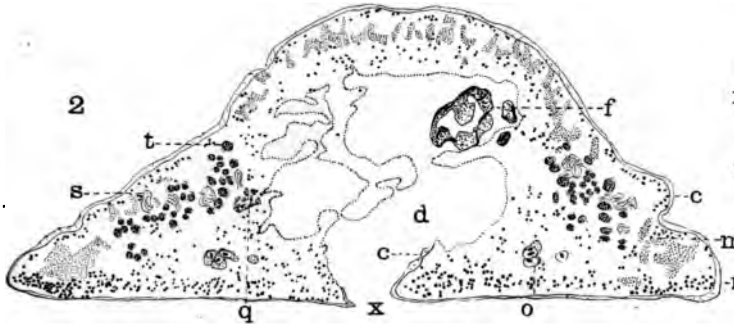

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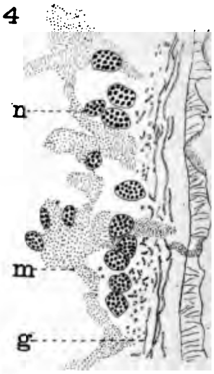

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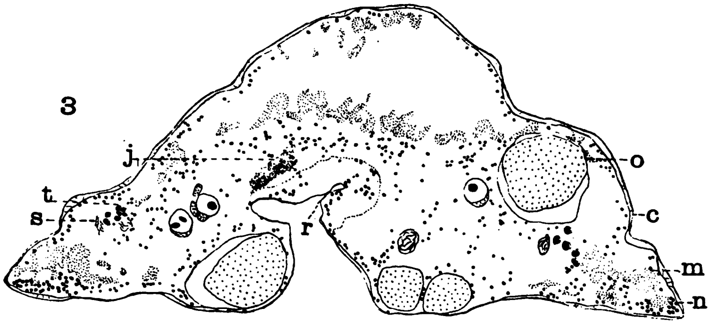

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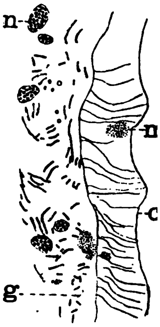

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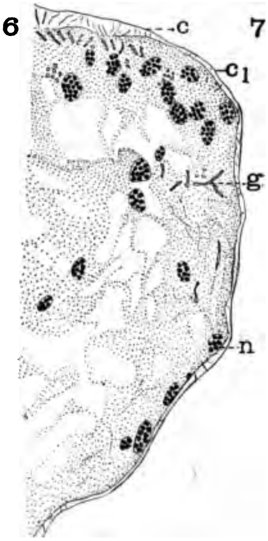

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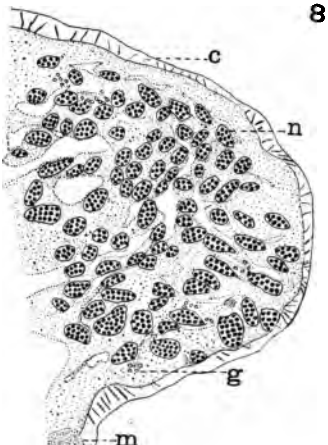

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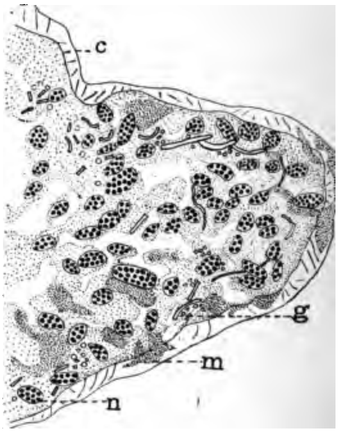

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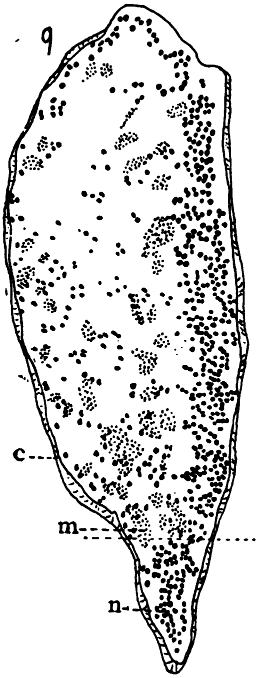

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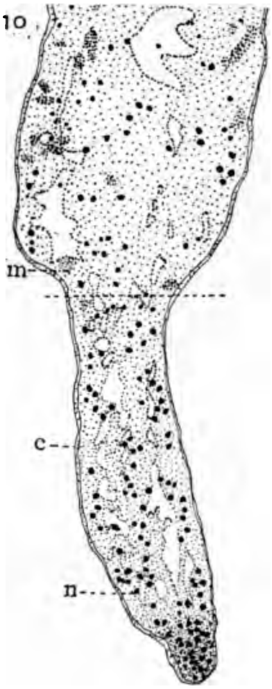

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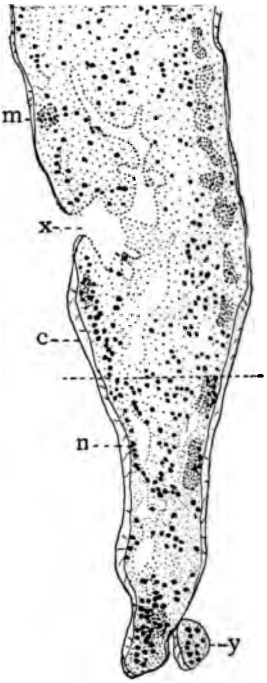

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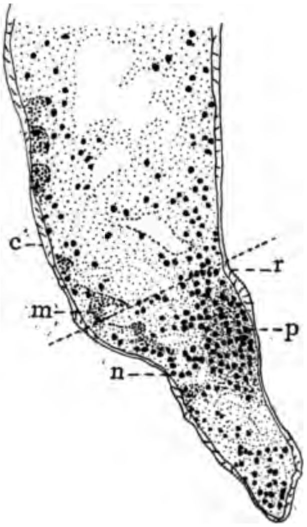

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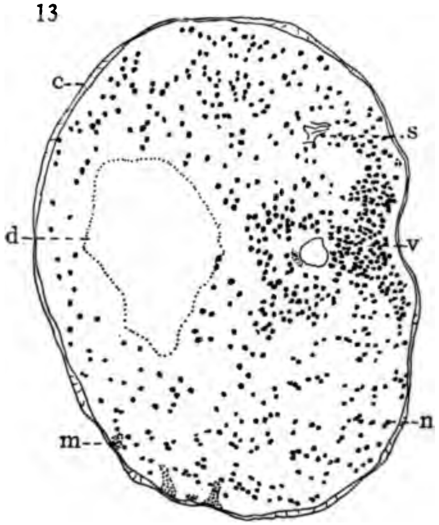

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STUDIES IN SPERMATOGENESIS

WITH ESPECIAL REFERENCE TO THE

"ACCESSORY CHROMOSOME"

BY N. M. STEVENS.

WASHINGTON, D. C.:
Published by the Carnegie Institution of Washington
September, 1905.

CARNEGIE INSTITUTION OF WASHINGTON

PUBLICATION No. 36

FROM THE PRESS OF
THE HENRY E. WILKENS PRINTING CO.
WASHINGTON, D. C.

STUDIES IN SPERMATOGENESIS WITH ESPECIAL REFERENCE TO THE "ACCESSORY CHROMOSOME."

By N. M. Stevens.

In connection with the problem of sex determination it has seemed necessary to investigate further the so-called "accessory chromosome," which, according to McClung ('02), may be a sex determinant. This view has been supported by Sutton ('02) in his work on *Brachystola magna*, but rejected by Miss Wallace ('05) for the spider.

The forms selected for study have been taken from several groups of insects, and are all species whose spermatogenesis has not been previously worked out. They are (1) a California termite, *Termopsis angusticollis;* (2) a California sand-cricket, *Stenopelmatus;* (3) the croton-bug, *Blattella germanica;* (4) the common meal-worm, *Tenebrio molitor;* and (5) one of the aphids, *Aphis œnotheræ.*

A brief account of a chromatin element resembling the accessory chromosome in *Sagitta* has been added for comparison. The spermatogenesis of each form will be described in detail, and a general discussion of the results and their relation to the accessory chromosome and sex determination will follow. The spermatogenesis of the aphid has been included in another paper, but a summary of results and a few figures will be given here for reference in the general discussion.

METHODS.

The testes were fixed in various fluids—Flemming's strong solution, Hermann's platino-aceto-osmic, Gilson's mercuro-nitric, Lenhossek's alcoholic sublimate acetic, and corrosive acetic. Flemming's and Hermann's fluids followed by safranin gave good results in most cases. The mercuro-nitric solution and Lenhossek's fluid gave excellent fixation and were preferable to the osmic mixtures when it was desirable to stain the same material with iron-hæmatoxylin, and also with various anilin stains.

Heidenhain's iron-hæmatoxylin, either alone or with orange G or erythrosin, was used more than any other one stain. With osmic fixation safranin gave better results in some cases, because of the abundance of spindle fibers and sphere substance which were stained by hæmatoxylin. The safranin-gentian combination used by Miss Wallace and others in the study of the accessory chromosome did not prove to be especially helpful with these forms. Thionin was found to be a very useful stain for distinguishing between the accessory chromosome and an ordinary nucleolus. Licht-grün was often used in combination with safranin.

RESULTS OF INVESTIGATIONS.

Termopsis angusticollis.

In the termite it was not found to be practicable to dissect out the testes. The tip of the abdomen was therefore fixed and sectioned, young males whose wings were just apparent being used. The cells are all small, and could not be studied to advantage with less than 1500 magnification (Zeiss oil immersion 2 mm., oc. 12).

In the spermatogonium there is a very large nucleolus (plate 1, fig. 1), which in the iron-hæmatoxylin preparations is very conspicuous, but does not stain like chromatin with thionin or other anilin stains, nor does it behave like an accessory chromosome during the maturation mitoses. Before each spermatogonial division it divides as in figures 2 and 3, and the same is true for each maturation mitosis. Figure 4 shows the 52 chromosomes of a spermatogonial division in metaphase. Figures 5 and 6 are young spermatocytes, showing the division of the nucleolus. Figures 8, 9, and 10 show a stage immediately following that shown in figure 6 and evidently persisting for some time. The spireme thread is very fine, stains deeply, and is wound into a dense ball, often concealing one (fig. 10) or both nucleoli (fig. 8). Figure 11 shows the next stage; the bivalent chromosomes are so disposed as to give the familar "bouquet stage," with the loops directed away from the centrosome and sphere (*c*). Figures 12, 13, and 14 show the later development of the same stage, the chromatin loops becoming thicker by the concentration of the smaller granules to form the larger ones seen in figure 14. The loops now straighten out and extend in various directions across the nuclear space (figs. 15, 16, 17). In fig. 18 *a* a longitudinal split is seen in several chromosomes. Figures 18 *b*, 19, 20, and 21 show various stages in the contraction of these split bivalent chromosomes to form diamond-shaped tetrads, each side of which is a univalent daughter chromosome. The tetrads come into the spindle in this form (figs. 22, 23), and change to the form shown in figure 24 during the metaphase (figs. 22, 26, 28). *Figures 25 and 27* show the 26 bivalent chromosomes, or tetrads, in early and late metaphase, respectively, and figures 29, 30, and 31 in anaphase. This is certainly a reduction division, for the tetrads are always somewhat elongated and come into the spindle with their longer axes parallel with the axis of the spindle. The aberrant bodies in these figures are probably remains of the nucleoli; they are found only in iron-hæmatoxylin preparations. Figures 31 and 32 show exceptional cases where the cell has divided. Usually the two daughter nuclei are formed in an undivided cell. The resting-stage between the two divisions is only partial. The nucleolus appears and divides into two (figs. 33–36), and the chromosomes change into the dyad form (fig. 36), in which they come into the second maturation spindle (figs. 37, 38). The equatorial plate again shows 26 chromosomes (fig. 39), The formation of the spermatozoa is peculiar in that the original spermatocyte cell-body, as a rule, does not divide; but the four nuclei resulting from the two maturation divisions develop into sperm-heads in one cell. All have a nucleolus (fig. 41), and in a slightly later stage (fig. 42) the elongated nuclei have a distinct centrosome and sphere at the posterior end. Later stages are shown in figures 43, 44, and 45.

The points of greatest interest in the spermatogenesis of *Termopsis angusticollis* are, (1) the fact that no accessory chromosome is present; (2) that the method of tetrad formation and reduction are clear, despite the fact that the cells and the chromatin elements are quite small; and (3) the failure of the cell-bodies to divide and the consequent development of four spermatozoa in one cell.

Stenopelmatus.

The spermatogonium of *Stenopelmatus* contains from one to three large nucleoli, which stain much less with thionin than does the spireme (plate II, figs. 46, 47, 48). As the distinct chromosomes come into view in the prophase of mitosis, two are seen to be nearly twice as long as the others, but of equal length (figs. 48, 49, 50.) There are 46 chromosomes in the equatorial plate of a spermatogonial spindle (fig 50). Besides the nucleolus (*n*), there appears in the young spermatocyte a conspicuous element (*x*) which stains deeply with all chromatin stains (fig. 51). It is closely applied to the nuclear membrane and is connected with an end of the spireme (figs. 51–54). At first it is quite small, and it gradually increases in size during the spireme stage. There is no "bouquet stage" in this form. Figure 55 shows the spireme segmented and split longitudinally. The segments have begun to open out at the center to give the cross which is the typical tetrad form in *Stenopelmatus*. Figures 56, 58, 59, and 60 show various stages in the contraction of the split segments to form crosses and diamond-shaped rings. The tetrads usually remain connected by delicate linin threads, as shown in figures 57 and 60, also in figures 62 and 63, the latter taken from the metaphase of the first maturation spindle. If these linin connections persist, as they appear to do, from the segmentation of the spireme to metakinesis, the first division of the contracted tetrads must be longitudinal, corresponding to the split in the segments of figures 55, 57, 58, etc. The chromosomes in the metaphase usually appear as dumbbells (fig. 66) or elongated crosses (fig. 67), but occasionally one can be found which still shows its tetrad nature (fig. 64), so clearly indicated in the quadrivalent crosses of figure 59. In the anaphase the chromosomes are often split as in figure 68, and occasionally the two components can be seen as plainly as in figure 65. Figure 61 shows the various shapes assumed by the element x during the tetrad-stage of the chromosomes. This element x almost invariably appears in a vesicle near one pole of the spindle (figs. 67, 68); in exceptional cases it is found nearer the equatorial plate, as in figure 66, or even in the same plane with the ordinary chromosomes, but always somewhat isolated from them. In position and form this element resembles the accessory chromosomes described by Baumgartner ('04) for *Gryllus domesticus;* in its mode of origin it seems to differ from the other accessory chromosomes yet described.

Figures 69 and 70 show the 23 bivalent chromosomes in metaphase; in figure 69 the element x is shown partly behind the large chromosome and at a different level. In figures 66 and 67 the one exceptionally large chromosome doubtless represents the two larger ones of the spermatogonia. In the anaphase the element x is sometimes as conspicuous as in figure 71; in other cases it is concealed either behind or within the polar mass of chromatin. In this form there is a distinct resting stage between the two maturation mitoses (figs. 72–75). The element x is conspicuous in one-half of the cells (figs. 72, 73); it may be included in the nucleus as in figure 72, or it may be partly or wholly outside, as in figures 74, 75, and 76. In the latter case, but not in the former, it is surrounded by its own membrane. As the chromatin begins to condense for the second mitosis, disintegration of the element x becomes apparent. This is most easily made out in cases where the element is isolated, as in figures 75 and 76; but there seems to be little doubt that it disappears before the metaphase of the second maturation mitosis. It is not possible to count the chromosomes in this stage, they are so crowded together, but it is not probable that such a conspicuous chromatin element as that seen in the first division could escape detection, even if it were in the equatorial plate among the chromosomes. No aberrant element is ever seen in these spindles;

and, moreover, all of the spindles and all of the spermatids appear to be exactly alike at the same stage. The chromosomes are double in the prophase (fig. 77) and always appear double in the equatorial plate (fig. 78), the paired elements corresponding to those of figure 65.

In figure 80, plate III, a pair of spermatids is shown with nuclear membrane formed and the spindle fibers twisted in a characteristic manner. Figure 81 is a slightly later stage with the spindle-remains massed against the nuclear membrane. Curiously enough there appears in the nucleus of every spermatid a body similar to the element *x* of the spermatocytes of the first order (figs. 82–86). This body is often applied to the nuclear membrane and connected with the spireme (figs. 84–86). It decreases in size and finally disappears (figs. 88–91). The spindle-remains divides (fig. 83), and a small part of it (*a*) goes to form the acrosome at the apex of the head (figs. 85–92). The larger part is probably utilized in the formation of the tail, for it gradually disappears as the tail develops.

The centrosome which, although small, is conspicuous in each mitosis, is seen in figure 83 between the two parts of the spindle-remains, applied to the outside of the nuclear membrane. In figures 85, 86, and 87 the relation of the tail (or its axial fiber) to the centrosome is shown. In figures 87 and 88, instead of the small spherical centrosome of figures 83 to 86, we have a much elongated body, at first (fig. 87) applied for its whole length to the nuclear membrane, but later lying along one side of a middle piece (*m*), as shown in figure 89, and in a later stage in figures 90 to 92. The mature spermatozoön with its forked anterior end appears in figure 93.

The points of especial interest in the spermatogenesis of *Stenopelmatus* are the development of the aberrant chromatin element *x* during the growth stage of the spermatocyte of the first order, its distribution to one-half of the spermatocytes of the first order, its disappearance during the rest stage between the two maturation divisions, and the development of a similar, though smaller, element in all of the spermatocytes.

Blattella germanica.

Unlike the spermatogonia of *Stenopelmatus*, those of *Blattella* have both a faintly-staining nucleolus and a deeply-staining chromatin element (*x*), and moreover the two are always closely associated (figs. 95, 96). The number of chromatin elements in the equatorial plate of late spermatogonial mitoses is 23 (fig. 97). Later events indicate that one of the 23 is the element *x*, but it is impossible to distinguish it here. Figure 98 is a very early stage of the spermatocyte of the first order, showing the element *x* as a U-shaped body. The centrosome was also conspicuous in all of the cells of this group. The spireme here, as also in figure 99, is fine and closely interwound. In figure 99 and again in figure 100 the element *x* is joined to the spireme as it is throughout the spireme stage. In the "bouquet" or "polarized" stage the combined nucleolus and element *x* are always at one side of the group of loops and down very close to the base of the figure (figs. 101, 103). In figure 102 most of the loops are cut across. The stage shown in figures 104 and 105 is a later one than that just described. Here we have again a continuous spireme connected with the element *x*, making it seem improbable that the bivalent chromosomes are really separated in the bouquet stage. Figure 106 gives some of the variations in form of the combined nucleolus and element *x*. The last of the five figures was taken from a giant cell containing at least twice the usual amount of chromatin. In one giant cell four unusually large combinations of this kind were found, and a corresponding amount of chromatin in the spireme. In figure 107 one sees the spireme divided into segments still joined by linin bridges. In figure 108 similar segments may be seen, one of them showing a longitudinal split. The element *x* is present, but the nucleolus has disappeared. In many cases the split, if it appears at all, closes quickly and the chromosome bends in U-shape, as in figure 109, plate IV. This figure also shows two centrosomes (*c*). In other cases the split persists as in figure 110 and leads to the formation of crosses of a tetrad character (figs. 111, 112, 113), as in *Stenopelmatus* and many other insects. Figures 114 to 117 show later stages of the U-shaped chromosomes. Perfect rings are rare. All sorts of variations are seen, broad and narrow U-shapes, rings split at one point or the opposite points, a U split at the bottom (fig. 114), pairs of parallel rods (fig. 115), and occasionally rods constricted in the middle and showing a longitudinal split in each half, as in figure 116. Figure 117 shows different views of the split rings. Apparently all of these forms straighten out so that the two components of the bivalent chromosome stand end to end as dumbbells or compressed crosses in the metaphase of the first maturation spindle (figs. 123–125). The element *x* remains concentrated and more or less spherical in form. Figures 118–122 are equatorial plates, with *x* absent in figure 120, in the same plane as the 11 other chromosomes in figure 119, far to one side in figure 118, and near one pole of the forming spindle in figure 122. It is also shown in various positions with regard to the spindle in figures 123 to 126 and 128 to 132. In figure 125 it is apparently double, and again in figure 129. In figure 130 one lagging chromosome shows the dyad nature of the products of the division of the tetrad. In this form there can be no doubt that reduction occurs in the first spermatocyte division. The element x is very often concealed by the polar aggregation of chromatin, but it is sometimes as conspicuous as in figures 131 and 132. The spermatocytes of the second order go into a complete resting stage before they are completely separated, and one of a pair shows the element x, while it is lacking in the other (fig. 133). At the close of the resting stage the chromosomes appear as 11 pairs of rods of considerable length, which gradually shorten and thicken and usually bend at the center, forming U's or V's (figs. 134–138). In one stage these double U's look much like tetrads (fig. 138). The rods straighten again as they shorten still more (fig. 139), become more closely approximated, and finally form dumbbells, as in figure 141.

The element x is, of course, present in only one-half of these nuclei. In the equatorial plate, figure 142, it is absent; in figure 143 it is present, but can not be distinguished from the other chromosomes, while in figure 144 it is rendered conspicuous by its spherical form and isolated position. In only a few cases has it been possible to distinguish x in the spindle. Figures 146 and 147 show two of these cases where this element is clearly double and of different form from the other chromosomes. It is probable that it divides and so goes into one-half of all of the spermatids, as in McClung's typical cases of the accessory chromosome. Figure 145 shows the usual appearance of the other chromosomes in metaphase. The two spermatids of a pair are always alike so far as any evidence of the presence of the element x is concerned (fig. 148). Figure 149 is an exceptional case, where one chromatin element (possibly x) has evidently divided late and been left out in the cytoplasm; a smaller chromatin granule is also present in the cytoplasm of each spermatid. All of the spermatids, as in *Stenopelmatus*, develop a deeply-staining body, which, however, in this case is usually centrally located and often appears double (figs. 150–152).

The spindle-remains (*Spindelreste*) forms a very conspicuous body at one side of the nucleus in the spermatids, and occasionally a mass of chromatin, probably due to imperfect mitosis, is found near the spindle-substance (fig. 150). The mass of spindle-substance at first appears structureless, but soon assumes the condition shown in figures 150 to 152. In one individual many of the spermatids had two balls of spindle-material (fig. 152), and the resulting later stages were double-tailed (fig. 153). Figure 156 shows how the spindle-substance goes into the tail and gradually disappears as the tail lengthens.

The centrosome is evidently applied to the nuclear membrane, as in *Stenopelmatus*, and the middle-piece is developed in connection with it, as in figures 156–157, 154–155, 158–160. The element *x* of the spermatids gradually disappears (figs. 150, 159). An acrosome develops at the anterior end, the head condenses and lengthens, and we have the ripe spermatozoön (fig. 161). The tail is very long and is shown only in part.

Of the forms studied, *Blattella* alone has many degenerate spermatozoa. Some follicles have none, others a number varying perhaps from one-fourth to three-fourths of the whole number. No evidence of degeneracy was detected among the young spermatids up to the stage shown in figures 154–155, where a few like figure 162 were found. Most of the degenerate forms occur among the nearly ripe spermatozoa or in the sperm-ducts. Such are shown in figures 163 to 168. The chromatin is strangely broken up into irregular clumps, and probably no two of these degenerate sperm-heads can be found which are alike. The tails are always imperfect. The distribution and varying numbers of these degenerate spermatozoa make it impossible to interpret their condition as due to the absence of the accessory chromosome, as Miss Wallace does in the spider. The only probable explanation, it seems to me, is imperfect mitosis. Cases where more or less chromatin is left behind in the cytoplasm, especially in the first spermatocyte mitosis, are very common, and such cases as those shown in figures 149 and 150 are not rare. The giant cells, so far as I have been able to trace them, do not develop into spermatozoa.

The most important points are:

(1) The presence of the element *x* in the spermatogonia, closely associated with the nucleolus.

(2) The uneven number of chromatin elements in the metaphase of spermatogonial divisions.

(3) The connection of the element *x* with the spireme up to the stage where the spireme segments to form the bivalent chromosomes.

(4) The varied character of the tetrads, showing the first spermatocyte division to be a reducing division in the sense that it separates whole chromosomes.

(5) The fact that the element *x* fails to divide in the first maturation division, does divide in the second, but can not be traced beyond the equatorial plate of the latter mitosis.

(6) The similarity of all the normal spermatids, though one-half of them must contain the element *x*, the other half not.

(7) The varying and often large number of degenerate spermatozoa.

An attempt was made to determine the somatic number of chromosomes. The dividing cells of the follicles of young eggs seemed to afford the most favorable material, but even here there was so much overlapping of the ends of the chromosomes that it was impossible to be absolutely certain of the number. In the two most favorable cases 23 were counted (fig. 94). This differs from McClung's count for similar cases among the Orthoptera, and Sutton's for *Brachystola magna*. The eggs have so far resisted all efforts to learn what part the odd chromosome may play in fertilization.

Tenebrio molitor.

In the metaphase of all spermatogonial mitoses where it was possible to count accurately, 20 chromosomes were found, 19 large ones of approximately equal size, and 1 small spherical one (figs. 169, 170). There is nothing in the resting nucleus of the spermatogonia which would suggest either a nucleolus or an accessory chromosome. The chromatin stains well during the whole growth period of the spermatocytes, but it is impossible to separate the period into so definite stages as in most other forms.

In the youngest spermatocytes one finds occasionally a cyst containing cells with nuclei like those of figures 171 and 172, indicating that a brief "synapsis" or condensation stage occurs at the close of the last spermatogonial mitosis. During the greater part of the period the chromatin forms a heavy, irregular, and often segmented spireme (figs. 173, 174). Shortly before the first maturation division, such split segments as appear in figure 175 are sometimes found; some of these simulate tetrads with slender connecting bands between the paired elements. Again, one finds a few cases like figure 176, where the spireme is segmented into bivalent chromosomes, each component showing a longitudinal split. This figure also shows the small chromosome. Usually, however, the irregular and much tangled spireme (figs. 173, 174) condenses into a heavy segmented band variously disposed in the nucleus (fig. 177). This band soon separates into the bivalent chromosomes shown in figures 178 and 179, giving 9 symmetrical pairs and 1 unsymmetrical one (fig. 179 *s*) composed of the small chromosome and a much larger mate. In the prophase of the spindle, in rare cases, some of the chromosomes are longitudinally split and transversely constricted, forming tetrads (fig. 180), but more often they appear as in figure 181. The unequal pair appears in each figure at *s*. In the metaphase (fig. 182) it is the last to come into the equatorial plate, possibly because of its lack of symmetry. The smaller component of this pair is always directed toward the equator of the spindle. Figure 183 shows a small tangential section of a spindle in metaphase, containing the unequal pair and one equal pair. In figure 184 a polar view of a metaphase is shown, the unequal pair, which was somewhat below the others, being indicated by stippling. Figures 184 *a* and 185 show that the unequal components of the unsymmetrical pair, as well as the equal components of the symmetrical pairs, are separated in metakinesis, making this clearly a reduction division. Two polar plates are shown in figures 186 and 187, one containing 10 equal elements, the other 9 equal ones and 1 small one. The telophase is shown in figure 188. There is no resting stage, but the new spindle is formed from the remains of the old one, and the spindle-shaped mass of chromatin seen in figure 188 either passes into the center of the new spindle or becomes enveloped by it. The double chromosomes separate as in figures 189 and 190. Figure 190 shows the small dyad, and figure 189 an aberrant one which may be its mate. The spindle in both divisions is peculiar in having outside of the spindle proper a dense mass of fibers which, in osmic material, stain deeply with iron hæmatoxylin. These fibers are shown in all the figures from 174 to 196. Figures 191 and 192 are equatorial plates of the two kinds of spermatocytes of the second order, figure 191 showing the small chromosome. An early anaphase appears in figures 193 and 194, which show both the small and larger chromosomes in metakinesis. Figure 195 is a later anaphase containing the divided small chromosome. In figure 196 are shown the two polar plates of a spindle corresponding to that of figure 195, and in figure 197 the polar plates of a spindle in which 10 equal chromosomes have been divided. In *Tenebrio molitor* the spermatids are therefore certainly of two distinct kinds, so far as the chromatin content is concerned.

In most of the young spermatids, after the nuclear membrane has formed, there appears an isolated chromatin element, which corresponds fairly well to the large or to the small component of the unsymmetrical pair, separated in the first mitosis and divided in the second. The clear portion of the nucleus containing this isolated element is at first turned toward the spindle-remains (fig. 198), but before the tail appears either the whole nucleus or its contents have rotated 180° (fig. 199). Various stages in the development of the spermatid are seen in figures 200 to 203. The clear region and the isolated element finally disappear (fig. 202 *b*), and the chromatin breaks up into coarser and then into finer granules within the sperm-head. In the later stages the centrosome is clearly seen at the base of the head (fig. 203).

In order to determine, if possible, the value of the unsymmetrical *pair of chromatin elements*, very young ovaries and ovaries with eggtubes were sectioned and the chromosomes counted in the dividing cells of the egg-follicle (♀ somatic cells), and in dividing oögonia. In both cases 20 large chromosomes were found. Figure 207 is the equatorial plate from a female somatic cell of a young egg-follicle. Figure 208 *a* and *b* shows two sections of an oögonium in the prophase of mitosis. In order to determine the number and character of the chromosomes in the male somatic cells, several male pupæ were sectioned. As in the spermatogonia, 19 large chromosomes and 1 small one were found. Figure 204 shows the equatorial plate of a dividing male somatic cell, and figures 205 to 206 are daughter plates from a similar cell. (Three large chromosomes of the plate shown in figure 206 are in another section.)

From these facts it appears that the egg-pronucleus must in all cases contain 10 large chromosomes, while the spermatozoön in fertilization brings into the egg either 10 large ones or 9 large ones and 1 small one. Since the somatic cells of the female contain 20 large chromosomes, while those of the male contain 19 large ones and 1 small one, this seems to be a clear case of sex-determination, not by an accessory chromosome, but by a definite difference in the character of the elements of one pair of chromosomes of the spermatocytes of the first order, the spermatozoa which contain the small chromosome determining the male sex, while those that contain 10 chromosomes of equal size determine the female sex. This result suggests that there may be in many cases some intrinsic difference affecting sex, in the character of the chromatin of one-half of the spermatozoa, though it may not usually be indicated by such an external difference in form or size of the chromosomes as in *Tenebrio*. It is important that related forms should be studied in order to ascertain whether the same chromatic conditions prevail in other species of this genus or possibly in the Coleoptera in general.*

Aphis oenotherae.

The spermatogensis of *Aphis* has been fully described in another paper and will merely be briefly summarized here for the purpose of comparison with other forms.

The spermatogonia contain a large nucleolus, which gradually disappears in the prophases of mitosis (plate VII, figs. 209–211). The youngest spermatocytes closely resemble the spermatogonia (fig. 212). There is no bouquet stage and no such marked spireme stage as in many other insects. The true synapsis occurs, as shown in figure 213, by pairing of like chromosomes side by side. This conjugation of like chromosomes is followed by a stage in which they are massed together at one side of the nucleus (fig. 214). In these latter stages the nucleolus has entirely faded out and nothing suggesting an accessory chromosome is present. Figures 215 and 216 are equatorial plates of the first spermatocyte mitosis. There are 5 chromosomes of different sizes and shapes, and figure 216 shows each one double. The first division of the chromosomes, though apparently longitudinal, is evidently a separation of the elements paired in a preceding stage, and is therefore a reducing division.

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* Prof. E. B. Wilson has recently found a similar dimorphism in the spermatozoa of *Lygæus* and other of the *Hemiptera heteroptera.*

The anaphase of the same mitosis is shown in figures 217 and 218; it is peculiar in that one chromosome always divides more slowly than the others, the two elements hanging together at one end. In figure 219 are sister spermatocytes of the second order, the "lagging" chromosomes still connected. The second maturation division is seen in metaphase in figure 220 and in anaphase in figure 221. Figure 222 shows a young spermatid, the five chromosomes still preserving their characteristic form. Figure 223 is the equatorial plate of the first maturation division of the winter egg, showing the same form and size relations of the chromosomes as in the spermatocyte divisions. Figures 224 and 225 are equatorial plates of a polar spindle (fig. 224) and of a segmentation spindle (fig. 225) of the parthenogenetic egg, where 10 chromosomes are present, 2 of each of the sizes found in the sexual germ cells.

So far as an accessory chromosome or any other visible evidence of a sex determinant are concerned, the results are entirely negative. The conditions shown do, however, support Mendel's conception of the "purity of the germ-cells," and also afford evidence in favor of Boveri's theory of the individuality of the chromosomes.

Sagitta bipunctata.

In connection with these insect forms it is of interest to find in the spermatogenesis of *Sagitta* a body which stains like chromatin and behaves somewhat like the accessory chromosome. It is found in all resting stages of the spermatogonia, closely applied to the nuclear membrane (fig. 226). It divides before each spermatogonial mitosis (fig. 227) and, though not often discernible in the spindle, appears in the next generation. Figure 228 is the last spermatogonial mitosis, and figure 229 shows the element x, and the chromosomes paired at one pole of the spindle. During the various phases of the growth *stage (figs. 230–232)* the element x is again applied to the nuclear membrane. In the prophase of the first maturation division this element divides (figs. 233–234), and in metakinesis the two elements are found in various positions with regard to the spindle (figs. 235–237), often as conspicuous as in these figures, but sometimes concealed among the chromosomes. Before the spindle for the second division forms, this element divides again and one of the products goes into each spermatid (figs. 238–241).

As *Sagitta* is hermaphrodite, there would appear to be no question of sex determination by any special chromatic element. The size of the element *x*, its evident chromatic nature, its division before each mitosis, and its presence in mitosis and in the spermatids, with the same staining qualities as in the previous rest stages, certainly indicate some important function, either in the whole process of spermatogenesis or in the formation of the sperm-head, of which it finally becomes a part. In *Sagitta* this element certainly can not be regarded as a specialized spermatogonial chromosome, or as chromatin rejected from the spireme. No such element is present in the ovogenesis of *Sagitta* (Stevens, '03), nor has any been detected in connection with fertilization. It is certain that none is present in the first segmentation spindle of the egg.

GENERAL DISCUSSION.

THE "ACCESSORY CHROMOSOME."

The literature bearing on the "accessory chromosome" of McClung, the "small chromosomes" of Paulmier, and the "chromatin nucleoli" of Montgomery has been fully discussed by McClung in the paper entitled, "The accessory chromosome—sex determinant?" ('02), and will therefore be considered here only in its relation to the several forms studied. The present status of the question has been well summarized more recently by Montgomery under the heading "Heterochromosomes" in the paper, "Some observations and considerations upon the maturation phenomena of the germ cells."

Three theories as to the function of the "heterochromosomes" have been advanced: (1) That of McClung that they are sex-determinants, since in the forms which he has examined these chromatin bodies occur in only one-half of the spermatozoa, and the sex-character is the only character which divides the individuals of a species into two approximately equal groups. (2) That of Paulmier and Montgomery that they are degenerating chromatin. Montgomery regards them as "chromosomes that are in the process of disappearance in the evolution of a higher to a lower chromosome number." (3) That of Miss Wallace, who suggests that in the spider only the one out of each four spermatids which contains the accessory chromosome is capable of developing into a functional spermatozoön, while the other three degenerate, as do the polar bodies given off by the egg. McClung is inclined to believe that the accessory chromosome is an element common to all of the male reproductive cells of Arthropods, and probably to vertebrate spermatocytes as well ('02).

Of the insects considered in this paper *Aphis* and *Termopsis* have no "accessory chromosome" or "heterochromosome" of any kind. The fact that no males develop from the fertilized eggs of *Aphis* might be offered as a reason for its absence, but such an argument would not apply to *Termopsis*. The sex-character may indeed be represented in the chromatin of some one of the pairs of paternal and maternal chromosomes of the spermatocytes, but there is no evident peculiarity by which one-half of the spermatozoa can be said to be different from the other half. As to McClung's statement ('02) "that if there is a cross-division of the chromosomes in the maturation mitosis, there must be two kinds of spermatozoa, regardless of the presence of the accessory chromosome," it appears to me that in a case like the aphid, where the paired elements of the five bivalent chromosomes are separated in the first maturation mitosis, there may be as many as seventeen kinds of spermatozoa instead of two. If, however, we suppose that the sex characters are segregated in the first maturation mitosis, there would, of course, be two equal classes of spermatozoa with reference to that character.

In *Stenopelmatus* the element x in certain stages closely resembles the "accessory chromosome" of McClung, and especially that described by Baumgartner for *Gryllus domesticus*, but its origin and fate are different. It first appears attached to an end of the spireme in the growth stage of the young spermatocytes, where it is much smaller than in later growth stages. It gradually increases in size, is a conspicuous element in the first maturation spindle, goes into one of each pair of spermatocytes of the second order, and there degenerates during the rest stage between the two maturation mitoses. The whole history of this element suggests that it may be rejected chromatin analogous to that observed in the ovogenesis of many forms. In *Sagitta*, for example, a considerable quantity of chromatin granules is given off by the chromosomes and cast out into the cytoplasm near the close of ovogenesis (Stevens, '03). Rückert ('92) has described a similar casting out of chromatin material by the chromosomes of the *oöcytes of Pristiurus*.

The spermatogenesis of *Stenopelmatus*, therefore, differs from that of the other Orthoptera which have been described in having (1) a larger number of chromosomes (46), (2) an even number in the spermatogonia, (3) an accessory chromatin structure in the spermatocytes of the first order, which disappears before the second maturation division.

In *Blattella* we have a typical "accessory chromosome," according to McClung. It appears (1) in all resting spermatogonia closely associated with a nucleolus, (2) in the spermatogonial mitoses as an odd chromatin element, making 23 in all, (3) in the growth stage of the spermatocytes connected with an end of the spireme and also with the nucleolus. It becomes separated from the other chromatin in the tetrad-stage, remains nucleolus-like in form, and later appears in the first maturation division either among the chromosomes or in a more or less aberrant position. It passes into one of each pair of spermatocytes of the second order, persists during the rest stage, appears in the second mitosis as a dyad and then divides, going into one-half of the spermatids. The spermatids, however, as in *Stenopelmatus*, all have the same appearance: each has in the center—not against the nuclear membrane—a small element that stains like chromatin. Occasionally a mass of chromatin is found outside the nucleus, but this is not constant enough to support the contention of Moore and Robinson ('05) that the "nucleolus" of the related form, *Periplaneta americana*, is fragmented and cast out into the cytoplasm. The spermatids all appear to develop equally well for some time, but as they approach maturity a varying proportion of them become degenerate. This can not, however, be due to absence of the accessory chromosome, as Miss Wallace supposes, in the spider; for in some follicles no degenerate spermatozoa are found, and in others more than half may be degenerate. All attempts to study fertilization stages of the egg have so far failed, and the chromosomes in the female somatic cells have not proved favorable for counting. Twenty-three have been counted in several cases, but there was always some chance of error. If 23 is the somatic number in both sexes, it must be maintained by union of sex-cells containing 11 and 12 chromosomes, respectively, the same unequal number occurring in the maturated eggs as in the sperm. Under such conditions it is difficult to see how the odd chromatin element of the spermatozoa can determine sex.

The brief description of the chromatin element *x* in *Sagitta*, introduced here because it behaves like the accessory chromosome in many particulars, serves as an example of the occurrence of such an element in the spermatogenesis of a hermaphrodite form, where it can not possibly be conceived of as a sex determinant. In *Sagitta* it is known to be confined to the male germ-cells. No such element occurs in the ovogenesis, in the sperm nucleus in the egg, or in the first segmentation spindle. Its function must, therefore, be confined to the process of spermatogenesis.

From the standpoint of sex determination, we have in *Tenebrio molitor* the most interesting of the forms considered in this paper. In both somatic and germ cells of the two sexes there is a difference not in the number of chromatin elements, but in the size of one, which is very small in the male and of the same size as the other 19 in the female. The egg nuclei of the female must be alike so far as number and size of chromosomes are concerned, while it is absolutely certain that the spermatids are of two equal classes as to chromatin content of the nucleus—one-half of them have 9 large chromosomes and 1 small one, while the other half have 10 large ones. Since the male somatic cells have 19 large and 1 small chromosome, while the female somatic cells have 20 large ones, it seems certain that an egg fertilized by a spermatozoön which contains the small chromosome must produce a male, while one fertilized by a spermatozoön containing 10 chromosomes of equal size must produce a female. The small chromosome itself may not be a sex determinant, but the conditions in *Tenebrio* indicate that sex may in some cases be determined by a difference in the amount or quality of the chromatin in different spermatozoa. This is much the most suggestive part of the work, and it will be followed up by the study of related forms.

There appears to be so little uniformity as to the presence of the heterochromosomes, even in insects, and in their behavior when present, that further discussion of their probable function must be deferred until the spermatogenesis of many more forms has been carefully worked out.

BRYN MAWR COLLEGE, *May 15, 1905.*

BIBLIOGRAPHY.

BAUMGARTNER, W. J.

'04. Some new evidences for the individuality of the chromosomes. Biol. Bull., vol. 8, no. 1

McCLUNG. C. E.

'99. A peculiar nuclear element in the male reproductive cells of insects. Zool. Bull., vol. 2.

'00. The spermatocyte divisions of the Acrididæ. Kans. Univ. Quart., vol. 9, no. 1.

'01. Notes on the accessory chromosomes. Anat. Anz., bd. 20, nos. 8 and 9.

'02. The accessory chromosome—Sex-determinant? Biol. Bull., vol. 3, nos. 1 and 2.

'02*a*. The spermatocyte divisions of the Locustidæ. Kans. Univ. Quart., vol. 1, no. 8.

MONTGOMERY, THOS. H., JR.

'01. A study of the chromosomes of the germ-cells of Metazoa. Trans. Amer. Phil. Soc., vol. 20.

'01*a*. Further studies on the chromosomes of the *Hemiptera heteroptera*. Proc. Acad. Nat. Sci. Phila. 1901.

04. Some observations and considerations upon the maturation phenomena of the germ-cells. Biol. Bull., vol. 6, no. 3.

MOORE, J. E. S., and ROBINSON, L. E.

'05. On the behavior of the nucleolus in the spermatogenesis of *Periplaneta americana*. Quart. Jour. of Mikr. Sci., n. s., no. 192 (vol. 48, part 4).

PAULMIER, F. C.

'93. Chromatin reduction in the Hemiptera. Anat. Anz., vol. 14.

'99. The spermatogenesis of *Anasa tristis*. Journ. of Morph., vol. 15.

RUCKERT, J.

'92. Zur. Entwickelungsgeschichte des Ovarialeies bei Selachiern. Anat. Anz., vol. 7, no. 4 and 5.

DE SINÉTY, R.

'01. Recherches sur la biologie et l'anatomie des phasms. La Cellule, vol. 19.

STEVENS, N. M.

'03. On the ovogenesis and spermatogenesis of *Sagitta bipunctata*. Zool. Jahrb., vol. 18.

SUTTON, W. S.

'02. On the morphology of the chromosome group in *Brachystola magna*. Biol. Bull., vol. 4, no. 1.

'03. The Chromosomes in heredity. Biol. Bull., vol. 4, no. 5.

WALLACE, L. B.

'00. The accessory chromosome in the spider. Anat. Anz., vol. 18.

'05. The spermatogenesis of the spider. Biol. Bull., vol. 8, no. 3.

WILCOX, E. V.

'95. Spermatogenesis of *Caloptenus femur-rubrum* and *Cicada tibicen*. Bull. Mus. Comp. Zool. Harvard Univ., vol. 27.

'96. Further studies on the spermatogenesis of *Caloptenus femur-rubrum*. Bull. Mus. Comp. Zool. Harvard Univ., vol. 29.

'97. Chromatic tetrads. Anat. Anz., vol. 14.

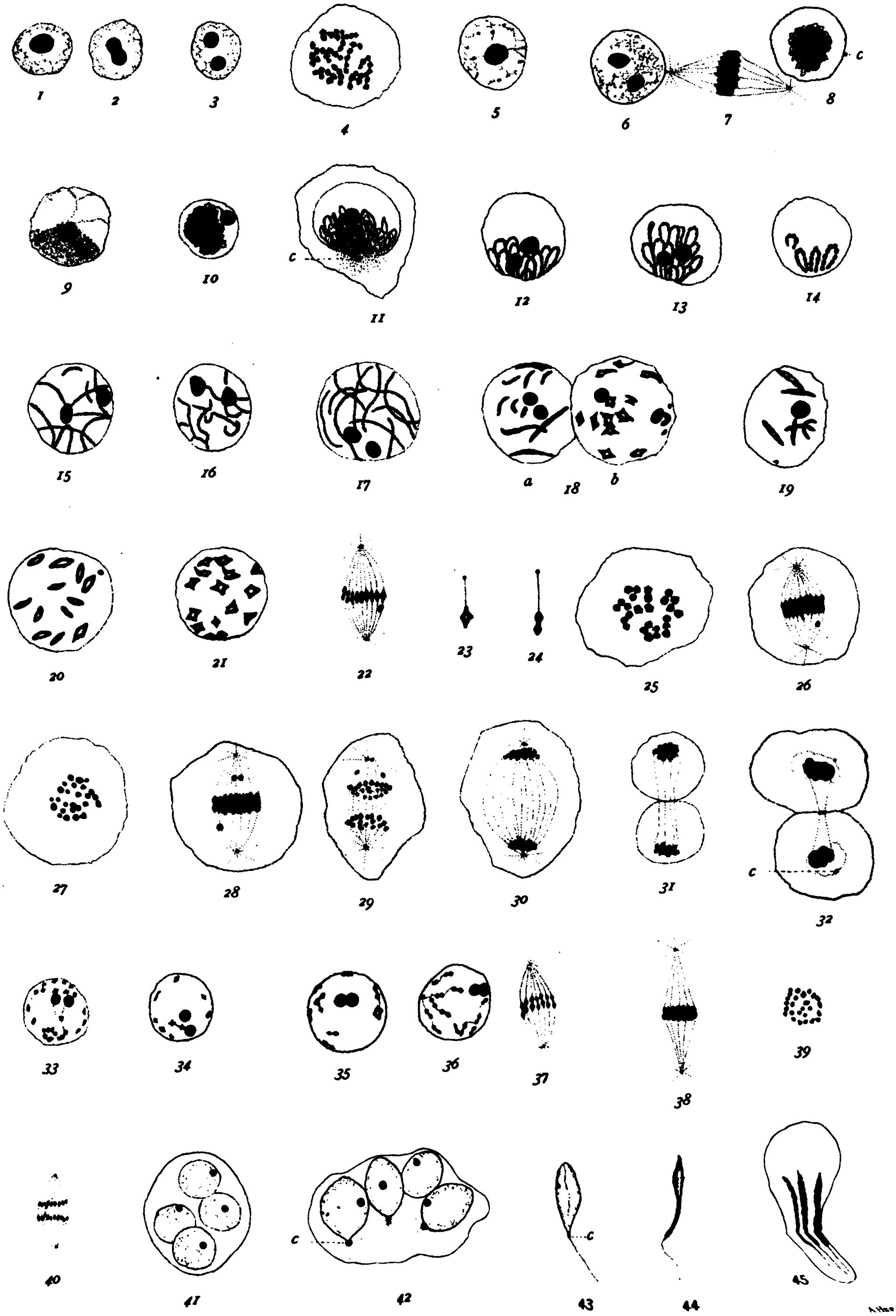
'01. Longitudinal and transverse division of chromosomes. Anat. Anz., vol. 19, no. 13.

[The figures of plates I-VI were all drawn with Zeiss oil-immersion 2 mm., oc. 12, and have been reduced one-third; those of plate VII with oc. 8, not reduced.]

PLATE I.

Termopsis angusticollis.

FIGS. 1-3. Resting nuclei of spermatogonia, showing division of nucleolus.
4. Equatorial plate of spermatogonial mitosis, 52 chromosomes.
5-6. Young spermatocytes, showing division of nucleolus.
7. First maturation spindle, and two nuclei (6 and 8) in same cyst.
8-10. Skein-stage—so-called synapsis-stage.
11-14. Bouquet-stage, showing two nucleoli, centrosome (*c*) in fig. 11, and loops made up of fine, then coarser granules.
15-17. Stage following preceding; loops straightened out and extending in various directions through nucleus.
18. *a*, Chromosomes much shortened and longitudinally split; *b*, chromosomes contracted to form diamond-shaped figures.
19. Stage between 18*a* and 18*b*.
20. Stage between 19 and 18*b*.
21. Stage similar to 18*a*, one chromosome in double diamond form.
22. First maturation spindle in metaphase, chromosomes in single and double diamond shapes.
23. Chromosome in single diamond or tetrad form, as they usually come into the spindle.
24. Double diamond-form assumed before metakinesis.
25. The 26 chromosomes of an early metaphase.
26. First maturation spindle in metakinesis.
27. Equatorial plate of first maturation spindle in metakinesis.
28. Another spindle, showing three granules which are probably remains of nucleoli.
29. Anaphase of first maturation mitosis, one centrosome divided.
30. Late anaphase.
31-32. Telophase, exceptional cases of division of the cell.
33-36. Partial rest stage between first and second maturation divisions, two nucleoli present. Chromosomes in fig. 36 in form of double diamonds ready for metakinesis.
37-38. Second maturation spindle in metaphase.
39. Equatorial plate of second maturation spindle, 26 chromosomes.
40. Same in anaphase.
41. Four spermatid nuclei in one cell, each nucleus containing one nucleolus.
42. A later stage, showing elongation of nuclei, centrosome and sphere at posterior end.
43-45. Later stages in the development of the spermatozoa, nucleolus grows gradually smaller.



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TERMOPSIS ANGUSTICOLLIS.

PLATE II.

Stenopelmatus.

FIGS. 46-47. Nuclei of spermatogonia, showing 2 and 3 nucleoli (n).

48-49. Prophase of spermatogonial mitosis, showing two exceptionally large chromosomes of equal length.

50. Equatorial plate of spermatogonial mitosis, 46 chromosomes.

51-54. Spermatocytes in spireme stage, nucleus containing a nucleolus (n), and a chromatin element (x), which is attached to one end of spireme and gradually increases in size during growth stage of spermatocytes.

55. Spireme longitudinally split and showing the beginning of cross formation.

56. Spireme segmented, tetrads forming.

57. One split segment and a part of another connected by bands of linin.

58. More open cross and diamond forms; element x conspicuous.

59-60. More contracted cross and diamond-shaped tetrads; linin bands shown in 60, where element x is also present.

61. Different forms assumed by element x during tetrad stage (figs. 56-60)

62-63. Diamond-shaped and contracted cross-shaped tetrads from metaphase of first maturation mitosis, showing linin connections.

64. Diamond-shaped tetrad with spindle-fibers attached; a-a, probably halves of one univalent chromosome; b-b, halves of the other.

65. Dyad from anaphase of first maturation mitosis.

66-67. Metaphase of first maturation spindle, showing element x in different positions.

68. Late anaphase of same.

69-70. Equatorial plate of first maturation spindle, 23 chromosomes and element x below, in fig. 69.

71. Chromatin massed at poles of spindle; element x isolated at one pole.

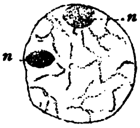
72-73. Two resting spermatocytes of the second order, one containing element x, the other not.

74-76. Successive stages of breaking down of element x.

77. Prophase of second division; dyads evident, but no sign of x in this or following stages.

78. Second spermatocyte division—metakinesis.

79. Same; late anaphase.



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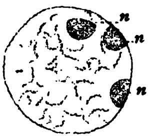

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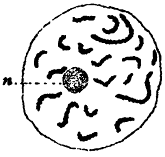

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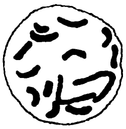
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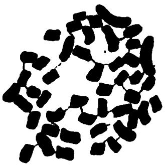
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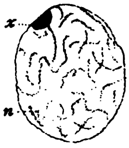

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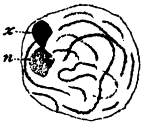

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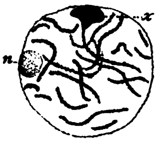

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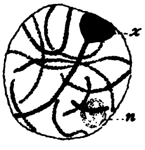

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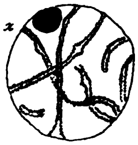

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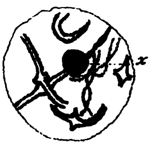

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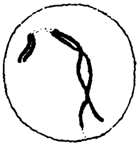
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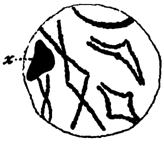

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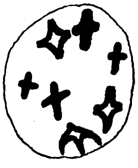
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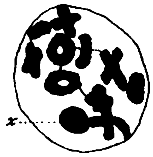

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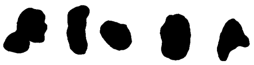
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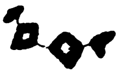
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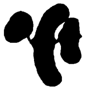
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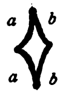

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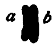

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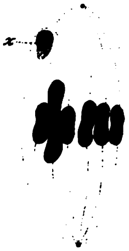

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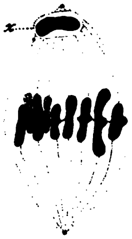

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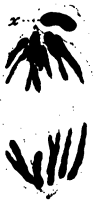

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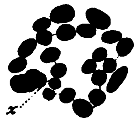

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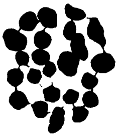
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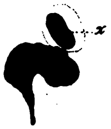

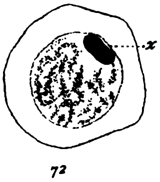

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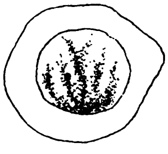
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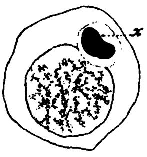

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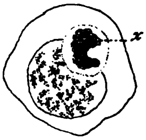

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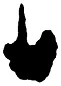
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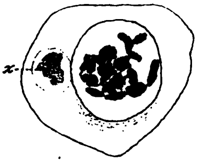

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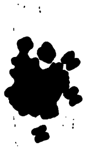
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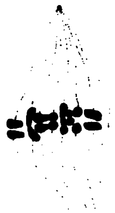
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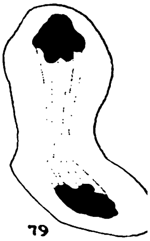
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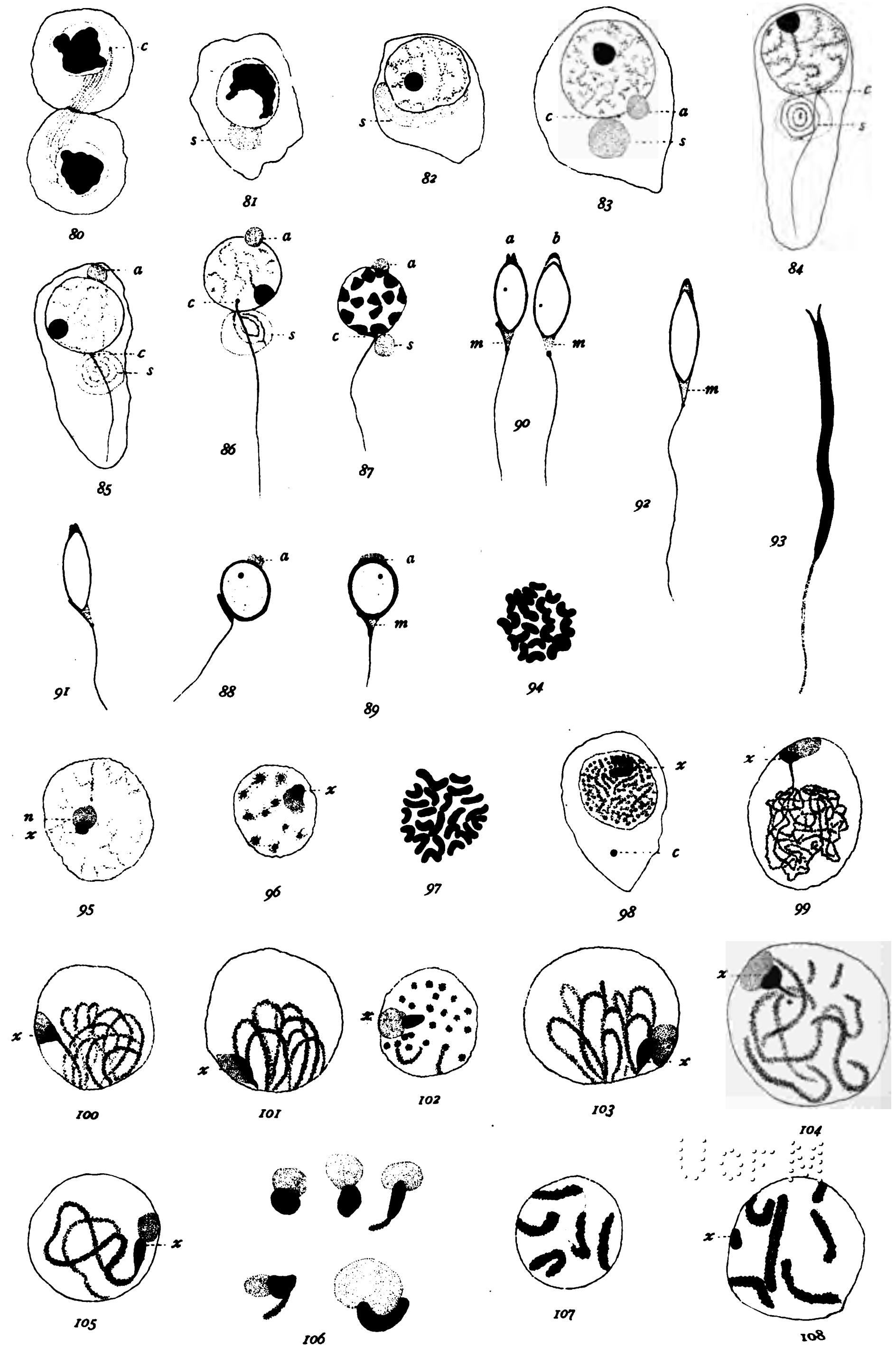
PLATE III.

Stenopelmatus.

FIG. 80. Telophase of second maturation mitosis.
81. Young spermatid, showing spindle-remains at *s*.
82. Spermatid showing a conspicuous chromatin element in nucleus, and spindle-remains (*s*) elongated.
83. Spermatid, showing centrosome (*c*) and divided spindle-remains (*s* and *a*).
84. Older spermatid, showing centrosome (c), axial fiber of tail, and spindle-remains (*s*).
85. Spermatid, showing acrosome material (*a*) migrating to side of nucleus opposite centrosome.
86. Slightly older spermatid.
87. Later stage of spermatid, showing condensed chromatin, elongated centrosome (*c*), acrosome material (*a*), and spindle-remains (*s*).
88-89. Older spermatids, showing formation of acrosome (*a*) and middle piece (*m*).
90-92. More advanced stages.
93. Mature spermatozöon.

Blattella germanica.

FIG. 94. Somatic cell from egg follicle, 23 chromosomes.
95. Spermatogonium, showing chromatin element (*x*) associated with a nucleolus (*n*).
96. Same, prophase of mitosis.
97. Equatorial plate of spermatogonial mitosis, 23 chromatin elements.
98. Young spermatocyte, showing centrosome (*c*) and U-shaped element (*x*).
99. Young spermatocyte, element *x* attached to one end of a long, fine spireme.
100. Coarser spireme stage.
101-103. Bouquet stage.
104-105. Later spireme stage.
106. Various forms assumed by the combined nucleolus and element *x;* last figure from a giant cell.
107. Segmenting spireme.
108. Similar stage to fig. 107, one chromosome longitudinally split; element *x* present.



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A.Hoen

STENOPELMATUS—BLATTELLA GERMANICA.

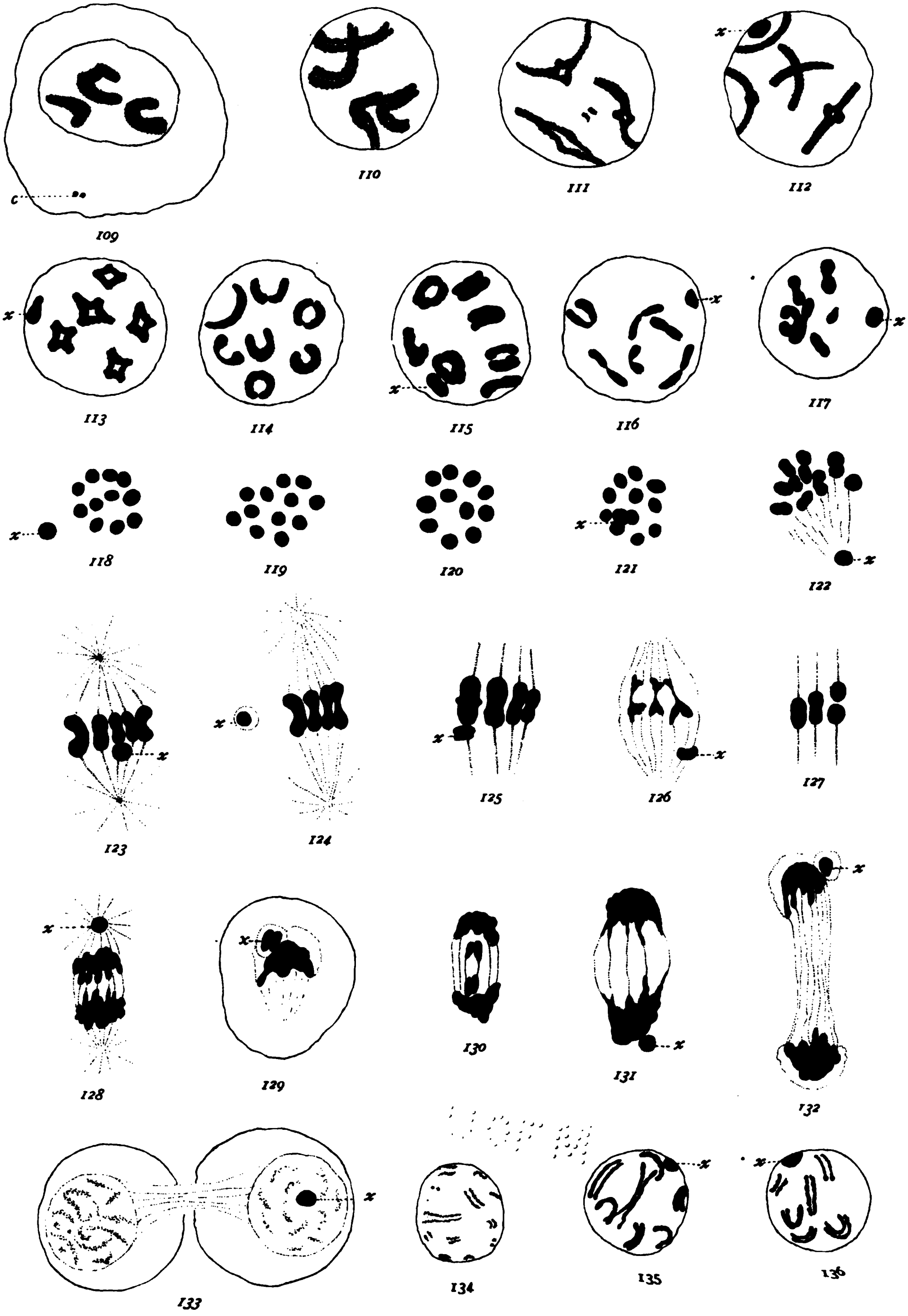
M 70 U

PLATE IV.

Blattella germanica.

FIG. 109. Similar stage to figs. 107 and 108; chromosomes U-shaped and not longitudinally split; two centrosomes present (*c*).
110. Longitudinally split chromosomes.
111-113. Various stages in formation of cross-shaped tetrads.
114-117. Bent rods, U-shapes, split rings, pairs of rods, and rod-shaped tetrads (116), which are equivalent to the crosses of figs. 112-113.
118-122. Metaphase of first maturation division, showing the element *x* in various positions.
123-127. First maturation spindle in metaphase.
128. Same in anaphase.
129-132. Late anaphase, showing element *x* double in 129, and a lagging tetrad in 130.
133. Telophase, with the element *x* in one daughter cell.
134-136. Prophase of second maturation mitosis, showing dyads and element *x*.

PLATE IV.



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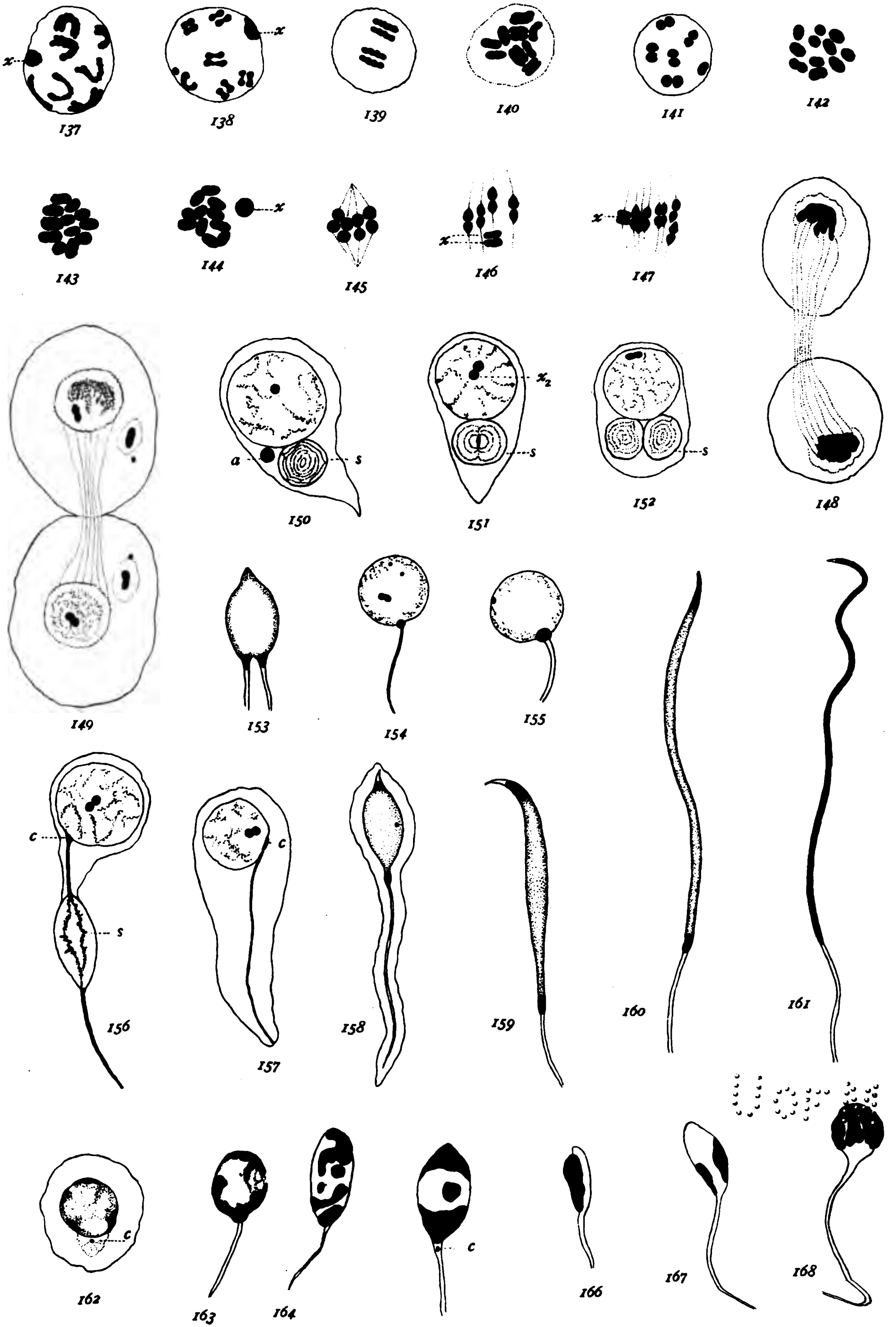
BLATTELLA GERMANICA.

PLATE V.

Blattella germanica.

FIGS. 137-141. Dyads contracting for second maturation mitosis.
142. Equatorial plate of second maturation spindle, containing 11 chromosomes.
143-144. Same, with 11 chromosomes and the element x.
145-147. Sections of second maturation spindles; element x dividing in 146 and 147.
148. Telophase of second mitosis.
149. Telophase of second mitosis, showing masses of chromatin left behind in cytoplasm.
150. Spermatid with extranuclear chromatin (a).
151. Similar stage; different view of spindle-remains (s) and of chromatin element (x_2).
152-153. Spermatid with divided spindle-substance and the corresponding double-tailed form.
154-155. Stages between 156 and 158.
156-157. Older spermatids than 151, showing spindle-remains (s) and centrosome (c).
158-160. Later stages in development of sperm-head.
161. Ripe spermatozoön.
162-168. Degenerate spermatids and spermatozoa.

STEVENS. PLATE V



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BLATTELLA GERMANICA.

PLATE VI.

Tenebrio molitor.

FIGS. 169-70. Equatorial plates of spermatogonial mitosis, showing 19 large and 1 small chromosome.

171-175. Condensation stage, bouquet stage, spireme stage, and rather rare tetrad stage of young spermatocyte.

176. Bivalent chromosomes, with longitudinal split; small chromosome shown at *s*.

177. Bivalent chromosomes condensed into a close spireme.

178-179. Bivalent chromosomes separating for mitosis. The unsymmetrical pair shown in fig. 179.

180. Prophase of first maturation mitosis, showing the unsymmetrical pair and the tetrad nature of the symmetrical pairs.

181. Prophase of same mitosis, showing symmetrical and unsymmetrical pairs, as in figs. 178 and 179.

182. Metaphase, unsymmetrical pair out of the equatorial plane.

183. Tangential section of a spindle in metaphase, showing the unsymmetrical pair and one symmetrical pair.

184. Equatorial plate of same mitosis, 10 chromosomes.

184*a*. Early anaphase, showing separation of the elements of the unsymmetrical pair.

185. Later anaphase.

186. Polar plate, showing 9 large and 1 small chromosome.

187. Polar plate, showing 10 large chromosomes.

188. Condensation stage between the two maturation divisions.

189-190. Prophase of second maturation division, fig. 189 showing 10 equal dyads, and fig. 190, showing 9 equal and 1 small dyad.

191. Equatorial plate, showing 1 small chromosome and 9 large ones.

192. Equatorial plate, showing 10 large chromosomes.

193-194. Tangential sections of spindle in metakinesis.

195. Anaphase of same mitosis.

196. Polar plates of a spindle, showing in each 1 small chromosome and 9 large ones.

197. Polar plates of another spindle, 10 large chromosomes in each.

198. Young spermatid, showing isolated small chromosome.

199. Young spermatid, showing isolated large chromosome and rotation of nuclear contents.

200-202*a*, *b*. Older spermatids.

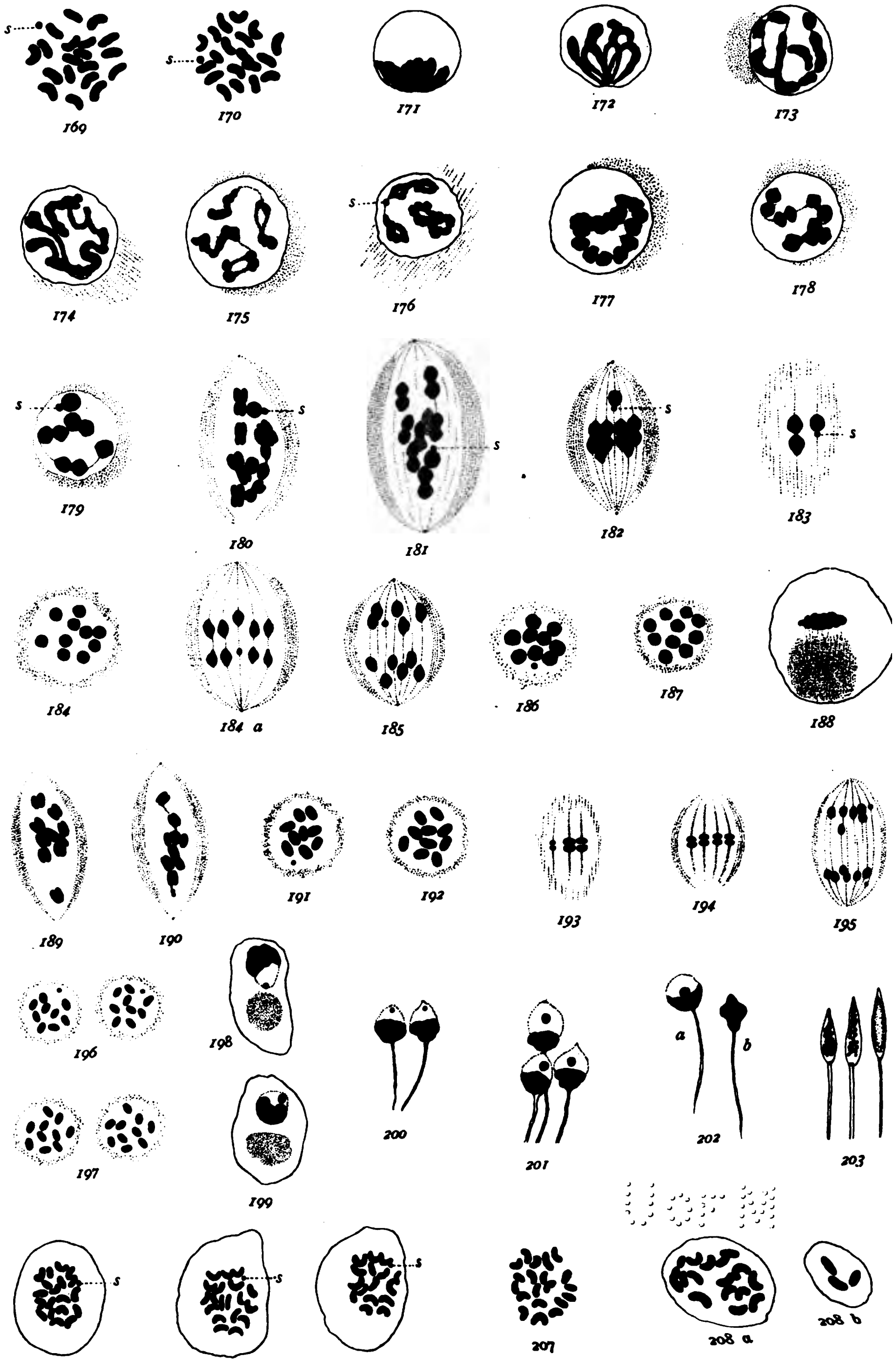
203. Sperm-heads, showing centrosome and granular chromatin.

204. Equatorial plate from dividing somatic cell of male pupa, showing 19 large and 1 small chromosome.

205-206. Daughter plates of a similar spindle, showing small chromosome in each; three of the large chromosomes missing in 206.

207. Equatorial plate of a dividing cell of follicle of a young egg, showing 20 large chromosomes.

208. Prophase of mitosis in a young oögonium, showing 20 large chromosomes in two sections, *a* and *b*.



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TENEBRIO MOLITOR

Plate VII.

Aphis oenotherae.

Fig. 209. Spermatogonium.

210-211. Spermatogonia in prophase of mitosis.

212. Young spermatocyte of first order.

213. Spermatocytes of first order; conjugation of the chromosomes.

214. Condensation of chromatin—spermatocytes of first order immediately before mitosis.

215. Equatorial plate of first maturation division.

216. Same, side view, showing chromosomes double.

217-218. Anaphase of same mitosis.

219. Daughter spermatocytes of second order.

220. Equatorial plate of second maturation mitosis.

221. Anaphase of same.

222. Young spermatid.

223. Equatorial plate of first polar spindle of winter egg.

224. Equatorial plate of polar spindle of parthenogenetic egg.

225. Equatorial plate of segmentation spindle of parthenogenetic egg.

Sagitta bipunctata.

Fig. 226. Resting spermatogonia.

227. Prophase of spermatogonial mitosis.

228. Last spermatogonial mitosis, metakinesis.

229. Anaphase of same, showing synapsis of chromosomes at pole of spindle, and element *x*.

230. Resting spermatocyte of first order.

231. Bouquet stage.

232. Later growth stage.

233. Prophase of first maturation mitosis, some of the chromosomes split longitudinally.

234. Later stage, chromosomes condensing and element *x* dividing.

235-237. First maturation mitosis.

238. Division of element *x* between the two maturation divisions.

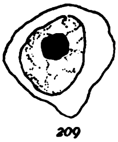
239. Second maturation mitosis.

240. Anaphase of same, showing the element *x* more deeply stained than the chromosomes.

247. Young spermatids; element *x* still conspicuous.

EVENS.

PLATE VII.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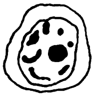
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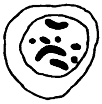
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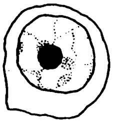
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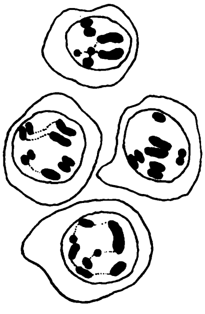
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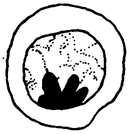
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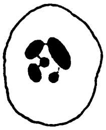
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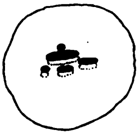
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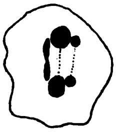
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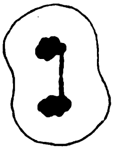
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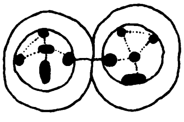
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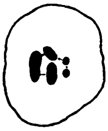
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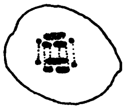
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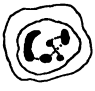
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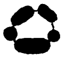
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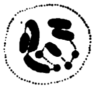
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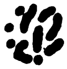
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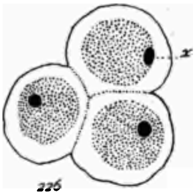

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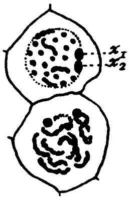

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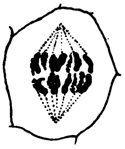
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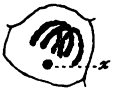

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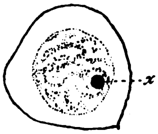

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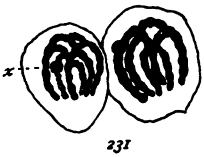

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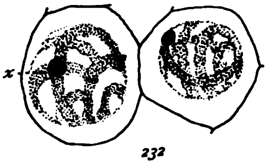

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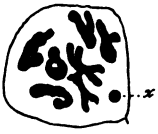

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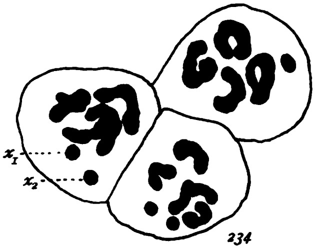

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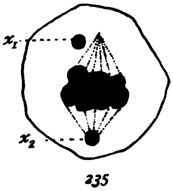

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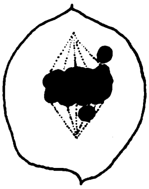
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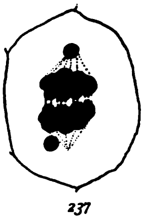
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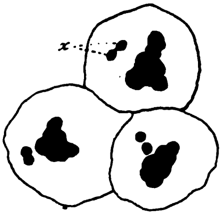

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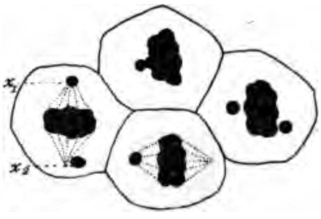

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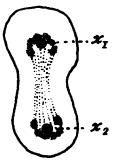

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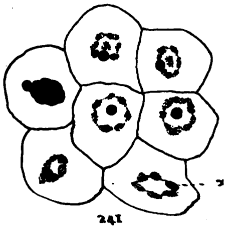

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N. M. S. del.

[From the BULLETIN OF THE TORREY BOTANICAL CLUB, 33: 93–99. 1906.]

The influence of moisture upon the formation of roots by cuttings of ivy

HARRIET RANDOLPH

When pieces of the leafy stem of English ivy (*Hedera Helix* L.) are kept for a time with one end immersed in water or in damp sand, roots generally arise on the part that is submerged. If the stem is then transplanted into soil, the new roots continue to grow and the piece of stem becomes an independent plant. This is the familiar method of raising new individuals from slips.

These new roots may arise close to the cut end of the stem and in any one or in all of the submerged internodes above the cut. They are white and thickly covered with root-hairs. Their structure in cross-section is shown in figure 3. When transplanted, the structure of the part subsequently formed in the soil is very like that of the part that grew in water.

The holdfast-roots, *i. e.*, the outgrowths along the side of the stem by which the ivy clings to the wall, under ordinary circumstances remain unchanged in the water. If kept there for a long time, the outer tissues decay and fall away, leaving the thicker-walled central part.

Experiments to be described below have shown that under varied conditions the ivy may send out roots that differ in some respects from these two kinds.

If the slips in water are kept in a greenhouse or in a room where the air is somewhat more moist than the normal, it sometimes happens that growth is set up in the submerged holdfast-roots. Delicate white outgrowths covered with root-hairs make their appearance either (1) from the tip of the holdfast-root, or (2) from some point along its side, or (3) from the matted felt-like complex formed by the growing together of several holdfast-roots as they attached themselves to a wall. (Figure 1, *hra*, showing near the lower end of the stem absorptive roots arising from the side of the holdfast mat that had adhered to the stone.) These secondary outgrowths from the holdfast-roots may occur in any of the submerged internodes and without any apparent relation to

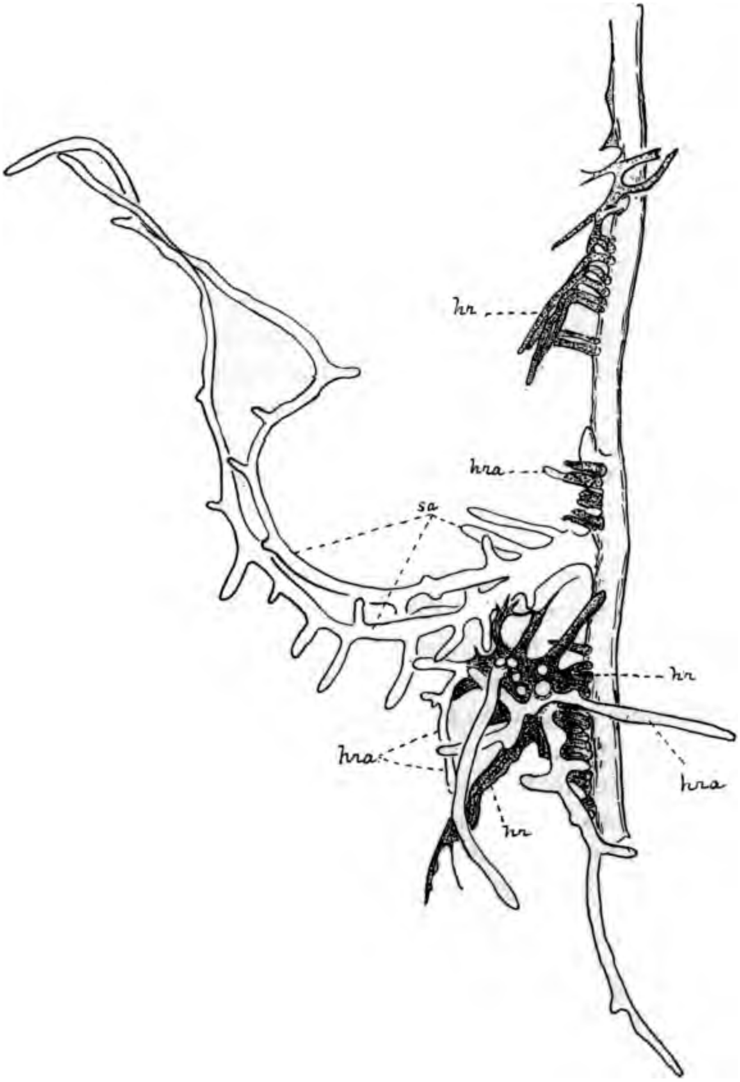

FIGURE I. Traced from a photograph of ivy stem grown in a greenhouse in air slightly moist, showing the part of the stem that was under water. The lowest group of holdfast-roots (*hr*) had grown together, forming a felt-like mat whose distal surface, which had adhered to a wall, is turned toward the observer. From this distal side of the mat of holdfast-roots absorptive roots (*hra*) have arisen. A group of absorptive roots springing from the stem (*sa*) slightly above has been turned aside in order to leave the view of the holdfast-roots and their outgrowths unobstructed.

the presence or absence of absorptive roots from the stem. The secondary roots increase in length and are not distinguishable in appearance from the ordinary roots that arise from the stem.

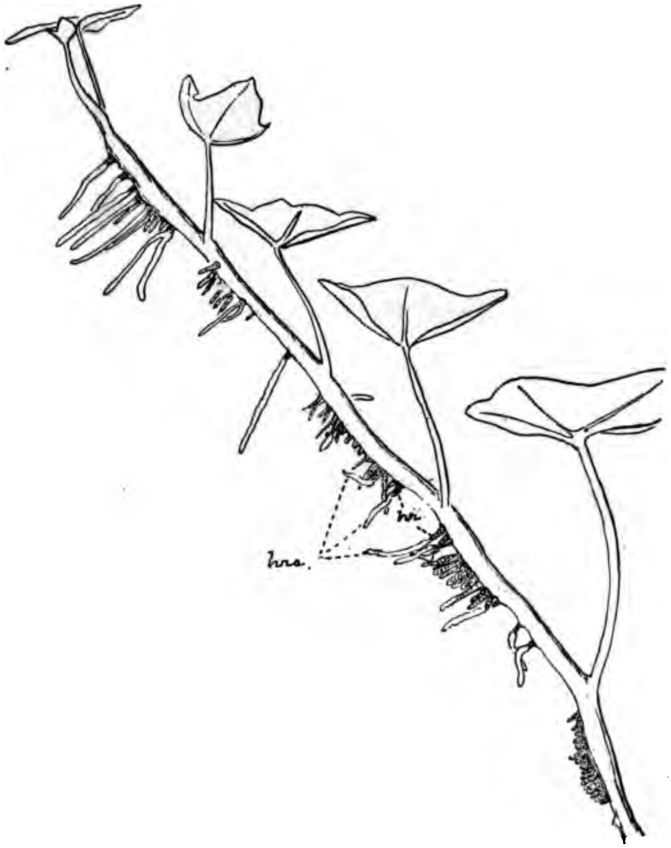

FIGURE 2. Traced from a photograph of young stems growing in water in air saturated with moisture, cut in May when the holdfast-roots had begun to appear. Many of the holdfast-roots (*hr*) have continued to grow, but the part grown in saturated air (*hra*) has a different structure from that of the original holdfast-roots. The holdfast-roots nearest the growing end of the stem had scarcely appeared when the stems were cut.

If the slips in water are kept in an atmosphere saturated with moisture, roots sometimes arise from the part of the stem that is in the air, above the surface of the water in which the stems are standing. They appear to arise indifferently on stems with leaves and on stems from which the leaves had been cut off at the beginning of the experiment. These roots, like those above mentioned that grow from holdfast-roots, have the same appearance as roots that grow from stems under water.

If young stems with green holdfast-roots beginning to grow out are kept in a vessel of water in saturated air, the growth of the holdfast-roots that are above the water continues, but the part that has grown in saturated air is white and covered with root-hairs.

In one instance, occurring in some slips growing in a pot of damp sand in the greenhouse, roots appeared on the sides of the stems about two centimeters above the sand and grew out and downward obliquely into it. They resembled the prop roots of Indian corn.

Thus under varying conditions the English ivy has seven classes of roots:

1. The roots of the seedling.
2. Holdfast-roots by which it clings to walls.
3. Roots arising from the stem under water.
4. Roots arising from the stem in an atmosphere saturated with water.
5. Roots arising from holdfast-roots under water.
6. Roots arising from holdfast-roots in an atmosphere saturated with water.
7. Roots arising from the stem over moist sand in greenhouse air.

I have not examined the structure of roots of class 1.

In the other roots the same kinds of tissues are present, but there are differences in the size or in the number of certain cells that give a characteristic appearance to sections of the different roots.

The structure in general is as follows: The epidermis consists of two (in one case of three) layers, with (or without) root-hairs; the cortical parenchyma is about five cell-rows in thickness; the endodermis surrounds the fibro-vascular bundles; and in the center there is a column of sclerenchymatous cells. Lying within the endodermis, next to it and also next or very near to the outer vessel of each bundle, are ducts lined by secreting cells. When the roots increase in thickness the ducts continue to lie next to the endodermis.

In the common roots that grow out in water from the stem (class 3) the parenchyma has very thin walls, and thus the epidermis and the endodermis are sharply differentiated from it. In these roots, and in the roots that grow out from them after transplantation into earth, the secreting cells are about the size of the cells of the surrounding tissue. The secreting cells contain dense protoplasm and the ducts are empty. The sclerenchyma of the central column is shaped in transverse section like a sharp-pointed star (figure 3).

In the following kinds of roots the ducts have contents * and the central sclerenchyma cells are more numerous and in section resemble a regular pentagon. Hence the phloëm is farther removed from the center of the stem and the endodermal ring is larger than in common water roots, etc.

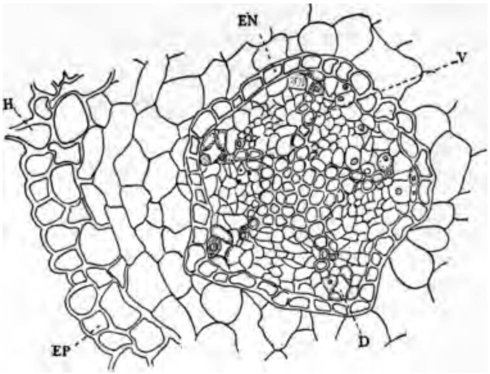

FIGURE 3. Cross-section of absorptive root springing from the submerged part of the stem of ivy in water. *d*, duct; *en*, endodermis; *ep*, epidermis; *h*, hair; *v*, vessels.

In the holdfasts (class 2) the epidermis is from two- to three-layered, and root-hairs are entirely absent or sparse (figure 4).

Adjoining or very near to each set of vessels of the holdfast-roots and on their peripheral side is a duct containing a yellowish substance and bounded in cross-section by four cells. This substance is probably the cement by which the holdfast-roots adhere to foreign surfaces.† Ducts are present in the cortical parenchyma of the stem, with contents apparently similar but with walls consisting of a greater number of cells (figure 5).

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* The roots here described were all preserved in Flemming's solution.

† DARWIN, C. Climbing plants 187, 188.

In roots arising from holdfasts (class 5) the secreting cells are from two to three times the size of the largest cells of that region, and their ducts, like those of the holdfasts contain a yellow substance.

In the roots arising from the stem in air saturated with moisture (class 4) the epidermis is without root-hairs, the secreting cells of the ducts are about four times the size of the largest of the neighboring cells, and the ducts contain a brownish-black substance.

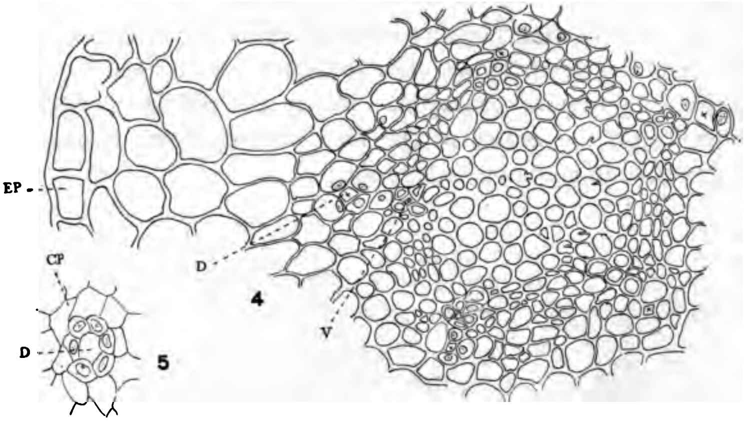

FIGURE 4. Cross-section of holdfast-root of ivy. *d*, the cavity of the duct (also marked ×); in some cases there are six sets of vessels; *ep*, epidermis; *v*, vessels.

FIGURE 5. From the stem of ivy, showing duct in the cortical parenchyma. It lies near the fibro-vascular bundles. *cp*, cortical parenchyma; *d*, duct.

In the corn-like roots which arise from the stem (class 7) the secreting cells are much larger than any of the surrounding cells, and the ducts (like those of the roots arising from the stem in air saturated with moisture) have brownish-black contents. Granules of this substance collected on the inner sides of the cell-walls bordering on the lumen were especially well seen in longitudinal section. These roots contain from six to nine vessels in a bundle, while in all the other kinds of roots the number varies from two to five.

The ivy, *Hedera Helix* L., is the typical example of dimorphism of shoot and leaf.* The possession of absorptive roots growing out into moist air from the holdfast-roots brings the ivy into relation with the tropical vines which send down roots to the soil from different points along the climbing stem. These roots (Nährwurzel) convey to the upper parts of the vines a supply of water for which their thin stems are wholly inadequate.* Roots of this kind arising from the stem are believed to have their phylogenetic origin from holdfast-roots (Haftwurzel).† The roots that now grow out from the holdfast-roots of the ivy may represent an early stage of this process. The presence of large numbers of root-hairs is evidence that these secondary roots that spring from the stem have the function of absorption. As in the case of the tropical vines, ‡ there is in the ivy a difference in structure between the absorptive roots and the holdfast-roots.

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*SCHENCK, H. Beiträge zur Biologie und Anatomie der Lianen 1: (SCHIMPER, A. F. W. Botanische Mittheilungen aus den Tropen 4:) 93, 94. 1892.

The presence of secondary absorptive roots in the ivy brings this species into closer relation with other members of its family (*Araliaceae*) in the tropics, and with epiphytes in general. Schenck § states: "In fast allen Familien oder Gattungen mit wurzelkletternden Lianen begegnen uns gleichzeitig auch epiphytische Formen. So enthalten die Araliaceen in den Tropen epiphytische Holzgewächse. . . . Wurzelkletterer und Epiphyten sind verwandte Vegetationsformen, und aus ersteren können sich leicht letztere herausbilden, falls die Verbreitungsmittel der Früchte oder Samen entsprechende sind. . . . In der That giebt es ja auch viele kreichende oder wurzelkletternde Epiphyten, welche in manchen Eigenthümlichkeiten mit den echten Wurzelkletterern übereinstimmen."

BRYN MAWR COLLEGE.

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* SCHENCK, *loc. cit.* 106.

† GOEBEL, K. Organographie der Pflanzen 488. 1900.

‡ GOEBEL, *loc. cit.* 487.

§ *Loc. cit.* 111.

The Effects of Compression on the Maturation and Early Development of the Eggs of Asterias forbesii.

By

Helen Dean King.

With plates I and II.

Eingegangen am 6. Dezember 1905.

In a few cases in which parthenogenesis normally occurs it has been shown that only one polar body is given off although the second polar spindle is formed and the chromosomes divide and migrate to the spindle poles as if in preparation for the second maturation division. The chromatin at the outer pole of the spindle remains in the egg and later unites with the chromatin at the inner pole (the egg-nucleus), appearing in this way to take the place of a spermatozoön. The main problem in mind when the experiments described in the present paper were undertaken was to determine whether, by subjecting eggs to pressure, it might not be possible to cause them to retain one or both of their polar bodies and thus to bring about their own parthenogenetic development. Another problem also presented itself in the same connection. If a compressed egg retains the chromatin that is normally extruded in the polar bodies, what will occur if the egg is then fertilized? Will all of the chromatin take part in the development, or will only that part of it representing the egg-nucleus unite with the sperm-nucleus? Whether normal development could take place under such conditions seemed also worth examining. The experiments were made at Woods Hole, Mass., during the summer of 1903, while I was occupying a research room of the Carnegie Institution in the Marine Biological Laboratory. I am indebted to Prof. T. H. Morgan, who suggested the problem, for assistance during the course of my investigations.

I. Material and Method.

In making the experiments, starfish were first thoroughly washed with fresh water in order that any spermatozoa adhering to their outer surface might be killed. The arms were then cut open, and if the eggs were ripe and in good condition they were at once put into a shallow dish containing fresh sea-water. A quantity of eggs was then transferred by means of a wide-mouthed pipette to a clean glass slide and covered with a large cover-glass supported at each edge and in the middle by narrow strips of thin paper. After twenty or thirty such slides had been prepared as quickly as possible with the aid of an assistant, the water was drawn from each by means of filter-paper until the eggs were greatly compressed. In one series of experiments eggs were kept under pressure for varying lengths of time (from ten minutes to four hours) in the hope that they would subsequently develop parthenogenetically; in a second series of experiments eggs were kept under compression until both polar bodies had been extruded from eggs of the same starfish that were developing normally in sea-water, they were then removed from compression and fertilized as were also the eggs of the control lot; in a third series of experiments a quantity of active spermatozoa was added to the water in which the eggs were put as soon as they had been taken from the starfish and the eggs were then compressed for several hours in order to determine whether fertilization and segmentation would take place. The conditions to which these eggs were subjected were, of course, very abnormal and consequently many of them died. The eggs that lived (not more than 40% in the most favorable cases) resumed their normal shape and size as soon as they were removed from compression and returned to sea-water. In some cases fertilized eggs developed in an apparently normal manner up to the blastula stage: unfertilized eggs divided in a few instances, but segmentation was very abnormal and did not go beyond the 8—16 cell stage.

Some of the eggs from each series were fixed at frequent intervals. The fluids used for fixation were corrosive-acetic (5% acetic acid) and picro-acetic, the former solution giving much the better preparations. The eggs were stained with iron-haematoxlyn which was followed in some cases by an overstaining with eurythrosin. I can see very little advantage in the use of the double stain, as the chromatin, centrosomes, and spindle fibres are brought out with great clearness by the use of iron-haematoxlyn alone.

II. The Maturation of Compressed Eggs.

The normal maturation of the starfish egg has been described by Wilson and Matthews (16) and by Hartmann (5). The account of this process as given by the former investigators is, in brief, as follows: The dissolution of the wall of the germinal vesicle of *Asterias forbesii* takes place at the point nearest the surface of the egg and here the centrosomes invariably appear, being found usually just outside of the nuclear membrane. The centrosomes soon become surrounded by a pronounced radiation and separate to become the poles of the first maturation spindle that develops from nuclear material and takes up a radial position at the periphery of the egg. The chromosomes of the first polar spindle are very small, rod-shaped structures and their number has not been definitely determined, although it is probably seventeen. After the second maturation division, the egg-centrosome and its aster completely disappear and the chromatin remaining in the egg forms a group of four or five small vesicles that later unite into the egg-nucleus. All that Hartmann adds to this description is that the chromosomes of the first polar spindle are evolved from the nucleoli.

The time required for the maturation of ripe eggs that have been removed from a starfish and placed in sea-water varies considerably in different cases as other investigators have noted. Occasionally the germinal vesicle disappears within five minutes after the eggs have been put in water, and maturation is then completed within an hour: in most cases, however, maturation does not begin until about half an hour after the eggs have been removed from the starfish and in favorable cases, from 75—95 % of them will mature.

The processes leading to the formation of the first polar spindle are apparently normal in many of the compressed eggs, although they are always considerably delayed. The fact that different eggs were compressed in different planes will probably account in great measure for the many variations from the normal type of maturation that were found. After the disintegration of the germinal vesicle there sometimes appears but a single centrosome which does not divide to form an amphister but acquires an extensive radiation and becomes a conspicuous monaster (Fig. 1). The central portion of this monaster may appear granular or reticular, and it sometimes contains a small, deeply staining, rounded body which appears very similar to the innumerable granules scattered throughout the cytoplasm that stain black with iron-haematoxlyn. In all cases where a monaster is found the chromosomes are massed at one side of it as in Fig. 1, thus producing structures strikingly like those found by WILSON (14, 15) in etherized eggs of *Toxopneustes* where the sperm-aster failed to divide, and in unfertilized eggs of the same species after treatment with $MgCl_2$. Whether, in compressed eggs, the rays from the monaster become attached to the chromosomes at a later stage and form a »fan-shaped« spindle similar to those described by HERTWIG (7), by M. BOVERI (1), and by WASSILIEFF (13), I have not been able to determine. Judging from the changes that take place in eggs of other species of echinoderms that have been subjected to abnormal conditions, I am inclined to think that such a monaster very soon disappears and makes no attempt to form a spindle or to become connected with the chromosomes.

In many cases, after the disappearance of the germinal vesicle, the chromosomes are found to be very unequal in size and somewhat irregular in form (Fig. 2). They may lie scattered in a granular mass which is presumably a part of the nuclear material (Fig. 2); or they may be massed closely together (Fig. 3), with no connection whatever with the spindle in either case. If the chromosomes are connected with the spindle they may be found at the equator (Fig. 6); or they may be scattered along the spindle fibres (Fig. 7); they may be massed at one pole (Fig. 11); or, as in Fig. 4, they may be found at the ends of the astral rays. In the latter case, the rays are probably concerned with the transportation of the chromosomes to the spindle fibres.

The polar spindles that are formed in these compressed eggs are usually fairly normal in shape, although they vary greatly in size as may be seen by comparing the spindle shown in Fig. 5 with that shown in Fig. 6. Both drawings, which were made under the same magnification, are from unfertilized eggs of the same lot that had been under compression for nearly two hours when they were fixed. In Fig. 5, not only is the spindle much larger than normal, but the polar asters are correspondingly large: the radiation of the asters is very extensive, and the central portions appear granular and contain distinct centrioles. In the two sections of the spindle I was able to count 63 separate chromosomes and it seems very probable, therefore, that the chromosomes have already divided for both maturation divisions. Another instance in which the chromosomes have apparently undergone a double division while on the first polar spindle is shown in Fig. 8, which was drawn from an unfertilized egg in which the spindle lay a considerable distance beneath the surface. The more usual types of polar spindles found in compressed eggs are shown in Figs. 6 and 7. Apparently, in these cases, the eggs were compressed in such a plane that the spindles were prevented from assuming a radial position, for both of them lie nearly parallel to the surface of the egg and some little distance beneath it. At the spindle poles are well developed asters, each with a spongy centrosome but no central granule. In Fig. 6, a number of rod-shaped chromosomes are found at the equator of the spindle and there is also a large, irregularly shaped, deeply staining body present which is presumably a portion of the nucleolus that has not yet given off all of its chromatin material. A somewhat similar body is also seen in Fig. 7, but it is much smaller, stains less intensely, and is evidently in the process of absorption.

A very few eggs were found in which the first polar body had been given off, apparently, in a normal manner. It is very probable that in these cases the spindle formed with its longitudinal axis parallel with the compression plates, consequently there was no interference with the migration of the spindle to the surface of the egg or with the subsequent cutting off of the polar body. Figs. 9 and 10 show cases in which unsuccessful attempts were made to give off the first polar body. In Fig. 9, the spindle has disappeared and the chromosomes that have migrated to the outer pole have fused into a solid mass lying close against the central portion of the aster; while at the inner pole the chromosomes are still distinct and are scattered over a considerable area. Fig. 10 is a drawing of a part of a section of an egg from the same series as that from which Fig. 9 was taken. In this instance the outer pole of the spindle is seen to extend into a pronounced elevation at the surface of the egg, but all of the chromosomes are collected near the inner pole where they appear much as do the chromosomes shown in Fig. 9.

In many cases the chromosomes are unequally distributed to the spindle poles (Fig. 18) and occasionally, as in Figs. 10 and 11, one pole receives all of the chromatin. The massing of the chromosomes at one pole of the spindle is not an uncommon phenomenon in echinoderm eggs that have been subjected to abnormal conditions. It has been found by Boveri (2) in non-nucleated fragments of the eggs of *Echinus microtuberculatus* that have been fertilized with spermatozoa of *Strongylocentrotus lividus*; by Morgan (11) in eggs of *Sphaerechinus* that have been shaken; by Wilson (15) in eggs of *Toxopneustes variegatus* that have been subjected to the influence of ether; and by Ziegler (17) in eggs of *Echinus microtuberculatus*. In all of these cases the phenomenon occurred in the segmentation-spindle of the fertilized egg. In compressed eggs in which there has been an unequal distribution of the chromosomes to the spindle poles I have usually found an unequal formation of the polar asters. The greater number of chromosomes is always massed at the larger pole, and it seems probable, as suggested by Ziegler, that the larger pole is the stronger of the two and can therefore draw more of the chromosomes to itself.

Although the first polar body may be extruded by eggs under compression, I have never succeeded in finding a second polar body. Apparently the chromosomes remaining in the egg after the first maturation division at once form one or several nuclei and no attempt is made to give off a second polar body. In Fig. 12, which was drawn from a fertilized egg that had been under compression for about an hour and a half when it was fixed, the first polar body is shown as a rounded structure containing a number of separate chromosomes. Directly under it, lying side by side, are two small, rounded nuclear vesicles in which the chromosomes are still distinct and are connected by a faintly staining reticulum. Between the nuclei is the aster from the inner pole of the first maturation spindle. Its centre is granular and the rays are not as prominent as they were at an earlier period. A section of another egg from the same series is shown in Fig. 13. Here also two nuclei have formed after the extrusion of the first polar body, one lying some distance below the other. Between the two nuclei is the inner aster of the spindle which has but few rays and an oval, granular centre containing two deeply staining granules. It is evident that in these two cases the chromosomes remaining in the egg after the extrusion of the first polar body separated into two groups. Whether a second maturation spindle formed I am not able to state, but as only one aster is present in either case I should judge that a separation of the chromosomes into two groups occured without the presence of a spindle, and that each group of chromosomes then formed a nucleus. In Figs. 12 and 13, one of the two nuclei represents the second polar body that has been retained in the egg, the other is the true egg-nucleus. Undoubtedly the nucleus that is close to the egg periphery in Fig. 13

must be considered as the second polar body; in most cases, however, it is quite impossible to distinguish between the two.

When two nuclei are formed after the first maturation division they are usually about the same size and they may contain from one to three deeply staining nucleoli as in Fig. 13. Often, however, as in Figs. 14 and 17, more than two nuclei are formed which are alike in structure although they may vary considerably in size. MORGAN (12) has found in eggs of *Arbacia* that have been subjected to the action of $MgCl_2$ that the size of the nucleus depends, to a certain extent at least, on the number of chromosomes it contains; and BOVERI (3), after a careful examination of the nuclei of plutei developed from non-nucleated fragments of the eggs of *Echinus* and also of »partially fertilized« eggs, has shown conclusively that in these cases the size of the nucleus is directly proportional to the number of chromosomes contained in it. Presumably, therefore, the difference in the size of the nuclei shown in Figs. 14 and 17 is the result of a separation of the chromosomes into unequal groups.

In a majority of eggs that continue their development under pressure no polar body is extruded and, after the dissolution of the germinal vesicle, the chromosomes at once form from one to five nuclear vesicles. The greater the number of these vesicles the smaller their size, yet their structure is the same in all cases and is apparently like that of the egg-nucleus in a normally developing egg. Each nucleus is round or oval and contains an irregular, granular network in which is imbedded one or more large nucleoli. The variations in the number of the nuclei in different cases can perhaps be explained as follows: only one nucleus forms under any of the three following conditions; first, if the polar spindle does not develop and all of the chromosomes come to lie closely massed together (Fig. 1); second, if the chromosomes remain together and fail to come into the spindle (Fig. 3); and third, if all of the chromosomes are collected at one pole of the spindle (Fig. 10). When the first polar body is extruded and the chromosomes remaining in the egg are equally divided into two groups, two nuclei of equal size are produced (Figs. 12, 13); if the chromosomes are unequally distributed to the poles of the maturation spindle which is unable to extrude the first polar body, then the two nuclei that form are of unequal size (Fig. 15); and if some of the chromosomes come to lie outside of the spindle, as in Fig. 18, several small nuclei of unequal size are formed (Fig. 17).

After a longer or a shorter period, varying considerably in different cases, every trace of a radiation disappears from the vicinity of the egg chromatin. Later, if several nuclei have formed they fuse into one large nucleus (Fig. 16) which migrates to the centre of the egg. All of the changes described up to this stage of development may take place in unfertilized eggs during the time that they are under compression. If unfertilized eggs are examined after they have been compressed for three hours, usually those that have matured are found to contain a single large nucleus in the centre of the egg. Several hours later some of these eggs may segment abnormally into an irregular mass of cells which bears little resemblance to a normal embryo. I have never found an unfertilized egg segmenting while under compression.

The results obtained by compressing unfertilized eggs of *Asterias forbesii* before the dissolution of the germinal vesicle are somewhat similar to those that LEFEVRE (7) has obtained by subjecting the unfertilized eggs of *Thalassema* to the action of various acids. LEFEVRE found that in some cases the polar bodies were not extruded and that maturation processes took place internally with no division of the cytoplasm. When this occured, four resting nuclei representing the egg-nucleus and the three polar bodies were usually found. Later these nuclei came together to form the cleavage-nucleus, the egg subsequently segmenting in an apparently normal manner and forming seemingly normal embryos.

III. The Fertilization and Segmentation of Compressed Eggs.

If active spermatozoa are added to the water in which the eggs are placed on their removal from the starfish and if the eggs are compressed for about an hour and then returned to sea-water usually a number of them segment regularly, but very slowly, up to the blastula stage. I have never succeeded in obtaining any older embryos.

In eggs of this series I have frequently found as great a difference in the size of the two pronuclei when they were about to fuse (Fig. 20) as has been found by VON ERLANGER (4) in eggs of *Sphaerechinus granularis*. This is not a common phenomenon in normally developing eggs of *Asterias forbesii*, and it must be due, in the eggs that have been subjected to pressure, to the fact that the egg-nucleus contains more chromation than the sperm-nucleus and is, therefore, correspondingly larger.

The segmentation-nucleus that is formed by the union of the two pronuclei appears to be very similar to that in normally developing eggs. In some cases there is an equal distribution of the chromatin to the poles of the segmentation-spindle, followed by an equal division of the cytoplasm. This is not invariably the case, however, as sometimes the segmentation-spindle lies quite close to the egg periphery and one pole of the spindle receives many more chromosomes than the other (Fig. 21); in such cases the cytoplasm divides unevenly and one cell is considerably larger than the other. Rarely, as shown in Fig. 22, all of the chromosomes go to one pole and there form a resting nucleus, the subsequent division of the egg results in the production of a nucleated cell and of one containing an aster and cytoplasm only.

Polyspermy is very frequently met with in fertilized eggs that have been subjected to pressure, and I have found as many as 18 spermatozoa in one egg. During the formation of the egg-nucleus most of the spermatozoa migrate towards the centre of the egg, each forming a sperm-nucleus and developing an aster which later becomes a small amphiaster. As a rule, all of the spermatozoa in an egg are in the same stage of development (Fig. 23). Occasionally, however, an egg is found containing spermatozoa that have not yet developed asters and also large sperm-nuclei with well formed amphiasters (Fig. 19). Apparently, in such cases, compression has so affected the egg that spermatozoa have been able to enter at various times, although normally, the entrance of one spermatozoön and the formation of the fertilization membrane effectually prevent polyspermy.

The egg-nucleus is readily distinguished from any of the sperm-nuclei by its larger size and by the fact that it never has an aster or any sign of a radiation near it. Several sperm-nuclei may unite with the egg-nucleus and later a triaster (Fig. 24), tetraster, or a more irregular form of multipolar figure may be produced, such as those that have been described by O. and R. Hertwig (6) and other investigators. Sperm-nuclei that do not unite with the egg-nucleus are resolved into chromosomes in situ at the same time that the segmentation-spindle is formed. Sometimes the chromosomes are evenly distributed to the poles of the small sperm spindles; in other cases all of the chromosomes go to one pole (Fig. 24). Isolated chromatin groups (Fig. 24) form later a number of chromatin vesicles *which subsequently* fuse into a resting nucleus. Occasionally cleavage takes place around a single sperm-nucleus and its aster as well as between the asters of the segmentation-spindle.

The segmentation of polyspermic eggs takes place at different times in the formation of the daughter-nuclei. Sometimes cleavage begins, as normally, while the chromosomes are moving towards the spindle poles; in other cases, segmentation may first be evident when the chromatin vesicles are forming; or it may be delayed until all of the vesicles have fused into the daughter-nuclei (Fig. 22). The eggs may divide simultaneously into 2, 3, 4 or more cells which are often very unequal in size. Occasionally an aster alone serves as a centre of division, and very rarely a portion of an egg which apparently contains nothing but protoplasm is constricted off from the rest. Through such irregular cleavage many kinds of abnormal cell groups are formed and it is not strange, therefore, that development never progresses beyond the early segmentation stages.

IV. The Development of the Eggs of Asterias forbesii when Deprived of a Normal Supply of Oxygen.

As it seemed possible that many of the results obtained in the experiments described above might be due, in part, to the fact that during compression the eggs had not been able to obtain a normal supply of oxygen, a second series of experiments were undertaken the past summer to determine the effects on the development of the eggs when they are placed in water deprived of the greater part of its oxygen. Loeb and Lewis (9) have shown that the eggs of the sea-urchin remain alive for many hours if they are placed in water from which all of the oxygen has been driven out by hydrogen or by other means and that they are capable of fertilization and of segmenting after being so treated for 64 hours. In making these experiments, Loeb and Lewis were endeavoring to prolong the life of the unfertilized egg, and they made no sections of the eggs to ascertain the internal changes taking place.

In making this series of experiments on the eggs of *Asterias forbesii*, small neck flasks were filled nearly full of fresh sea-water and then the water was boiled over an alcohol flamp from 25—35 minutes in order to drive out all of the air. The flasks were then sealed with rubber stoppers, and the neck of the flask and the stoppers were coated with paraffine to prevent the possible entrance of any air. After the water had cooled, a quantity of ripe eggs, just removed from the starfish, were put into the flask as quickly as possible and the flask again sealed as before. When the second polar body had been extruded from eggs of the same starfish that were maturing normally in sea-water, the flask was opened and the eggs contained in it were transferred into fresh sea-water. An examination of the living eggs at this time showed that in but a comparatively few cases had the germinal vesicle broken down, and that the second polar body had not been extruded in any case. Sections made of different lots of eggs that were fixed at short intervals for a period of several hours after their removal from the flask showed that maturation took place in a perfectly normal manner although it was greatly delayed. Not one of the hundreds of eggs that were examined segmented parthenogenetically.

In another series of experiments, pieces of testes containing active spermatozoa were placed with ripe eggs in a flask containing water that had been deprived of its oxygen. After the flask had again been sealed, it was shaken gently for some moments in order to liberate the spermatozoa and to facilitate the fertilization of the eggs. The eggs remained in the flask until large numbers of fertilized eggs from the same starfish, which were developing normally in sea-water, had segmented and were in the 2—4 cell stage; this was about three hours after the eggs had been removed from the starfish. When the eggs were transferred from the flask to fresh sea-water and examined, it was found that the germinal vesicle had broken down in a great many of the eggs and that a very few of them were in the 2-cell stage; none were in a later stage of development. Sections of eggs fixed at this time showed fully, in most cases, the formed segmentation-spindle. Polyspermy was not common and the eggs appeared perfectly normal in every respect.

An hour later, when many eggs of the control lot were in the 16-cell stage, the majority of eggs that had been deprived of a full supply of oxygen were just beginning to segment; some few were in the 4-cell stage. When the eggs of the control lot were in the late segmentation stages, the eggs experimented upon were mostly in the 8—16 cell stage, and some few of them were segmenting irregularly. The next morning, normal gastrulae were found in both lots of eggs; those of the control lot being somewhat further developed than the others.

In these experiments the eggs were, of course, not deprived of all oxygen as presumably a small amount was taken up by the water when the flasks were opened to admit the eggs. There can be no doubt, however, but that the eggs obtained much less oxygen than did those that were subjected to pressure; and in most of the experiments the eggs were under abnormal conditions for a much longer time than were the compressed eggs. The results obtained show that a deficiency in the supply of oxygen greatly retards the the development of the egg, but does not lead to a parthenogenetic development, to polyspermy, or to great irregularities in segmentation. Spermatozoa as well as the eggs are able to live for some hours in water containing but little oxygen; their activity is doubtless lessened, yet they can penetrate the egg and produce normal embryos. The abnormalities in development resulting from compression of the eggs cannot, therefore, be ascribed, even in part, to the lack of a normal amount of oxygen, but must be caused solely by the distortion of the egg and by the presence of an abnormal amount of chromatin: only the delay in the development of the egg can be considered as due, possibly, to a deficiency in the supply of oxygen.

General Conclusions.

Compression of the eggs of *Asterias forbesii* delays their development and usually leads to abnormal segmentation and early death. These results must be due, in the main, to the distortion of the eggs by pressure, and to the fact that in most cases one or both of the maturation divisions are suppressed causing a large amount of chromatin to be retained that normally would be ejected in the polar bodies. Apparently the cytoplasm of the eggs of *Asterias forbesii* has not the power of absorbing excess chromatin as is the case with the cytoplasm in the eggs of some other species, and, therefore, the presence of more than the normal amount of chromatin greatly interferes with development.

After a more or less successful attempt has been made to extrude the polar bodies, all of the chromatin remaining in the egg goes into the formation of one or several nuclear vesicles which later unite to form a large, rounded egg-nucleus. The egg-nucleus migrates to the centre of the egg and appears very similar to the one in a normally developing egg, although, as a rule, it is somewhat larger. The size of the egg-nucleus is not, however, directly proportional to the number of chromosomes it contains, as in eggs that have been subjected to pressure I have never found an egg-nucleus that was more than twice the size of the one in a normally developing egg. Both of the maturation divisions are frequently suppressed, and, therefore, the egg-nucleus in many cases must contain three times the amount of chromatin that is normally used in its formation. This result does not support BOVERI's (3) conclusion that »die Kernoberfläche der Chromosomenzahl direkt proportional ist«.

The majority of eggs that are fertilized either before or after compression are entered by several spermatozoa. Polyspermy in these cases cannot be attributed to the fact that the eggs were unable to obtain a sufficient supply of oxygen during the time they were under compression; for eggs placed in water deprived of oxygen are rarely entered by more than one spermatozoön, and polyspermy is no more common than is generally found to be the case whenever eggs are removed from a starfish and fertilized artificially. The presence in the egg of chromatin that is normally extruded in the maturation divisions can hardly be considered to be the cause of polyspermy, as spermatozoa frequently enter eggs before the first polar body is given off. It is probable, therefore, that polyspermy is commonly found in compressed eggs because the cytoplasm has been so affected by the compressure that it cannot offer any resistance to the entrance of the spermatozoa.

Not infrequently two, three, or even more sperm-nuclei fuse with the egg-nucleus and thus the segmentation-nucleus that is formed contains a vastly greater amount of chromatin than is normally present. In such cases it is obviously impossible that a normal combination of chromosomes should take place, and in consequence of the abnormal chromatin conditions the subsequent segmentation of the egg is very irregular. Polyspermy, even in eggs that have extruded both polar bodies, usually leads to abnormal segmentation as HERTWIG, WILSON, and others have noted: not infrequently such eggs divide simultaneously into three, four, or more cells as is the case in compressed eggs. When eggs have been subjected to pressure, segmentation may take place rapidly in one part and be greatly retarded in another part; and it is invariably that part of the egg containing the greatest amount of chromatin that is the last to divide. Apparently, therefore, an excess in the amount of chromatin delays the mitotic processes as well as causes irregularity in the time and manner in which they occur. The results obtained by compressing eggs of *Asterias forbesii* would seem to show that normal development is possible only when the chromatin conditions are normal.

Eggs of various species of echinoderms can be made to segment parthenogenetically by means of mechanical agitation, by the action of chloroform, ether, and various alkalies. It might be expected, therefore, that compression of the eggs which prevents one or both of the maturation divisions would have a like effect; the extra chromatin taking the place of a spermatozoön and bringing about a parthenogenetic development of the egg. Such, however, is not the case. In but very few instances did unfertilized compressed eggs segment, and when this occured the segmentation was very irregular and never progressed beyond the sixteen cell stage. It is probable that in such cases segmentation was not due to the presence of an abnormal amount of chromatin, but to a mechanical agitation of the eggs when they were transferred to fresh sea-water after compression. Even a slight jar occasionally causes unfertilized eggs to segment, especially if it occurs some little time after the eggs have been removed from the starfish, as MATHEWS (10) has shown. After an egg has received from a spermatozoön the stimulus for division, the presence of an excess of chromatin delays development, causes great irregularity in segmentation, and prevents the formation of a normal embryo.

Summary.

1) Compression of the eggs *Asterias forbesii* before the dissolution of the germinal vesicle usually prevents the extrusion of both polar bodies; occasionally one polar body is given off, but never two as is normally the case.

2) The retention of chromatin that is normally extruded in the polar bodies does not lead to a parthenogenetic development of the egg.

3) Polyspermy is very common in eggs that have been subjected to pressure, and as many as 18 spermatozoa may enter one egg. Several sperm-nuclei may fuse with the egg-nucleus and produce a triaster, a tetraster, or a more irregular form of mitotic figure.

4) In some instances compressed eggs that have been fertilized segment in an apparently normal manner up to the blastula stage; none develop beyond this stage. Abnormality in development is usually apparent at the first cleavage and frequently eggs divide simultaneously into three, four, or more cells. In all cases segmentation is greatly delayed.

5) The abnormalities produced by subjecting eggs to pressure are due in part to the distortion of the egg and in part to the presence of an abnormal amount of chromatin.

6) Deficiency in the supply of oxygen while eggs are under compression does not lead to abnormality in development. Ripe eggs placed in water deprived of oxygen maturate in a normal manner and, if fertilized, develop into normal embryos. These processes, however, take place much more slowly than in eggs developing normally in sea-water.

Bryn Mawr College, Bryn Mawr, Pa., November 27, 1905.

Zusammenfassung.

1) Kompression der Eier von *Asterias Forbesii* vor der Auflösung des Keimbläschens verhindert gewöhnlich die Ausstoßung beider Polkörperchen; gelegentlich wird ein Richtungskörperchen ausgestoßen, aber niemals zwei, wie es normalerweise der Fall ist.

2) Die Zurückbehaltung von Chromatin, welches normalerweise in den Richtungskörperchen ausgestoßen wird, führt nicht zu einer parthenogenetischen Entwicklung des Eies.

3) Polyspermie ist sehr gewöhnlich bei Eiern, die einem Druck unterworfen gewesen sind, und es können bis zu 18 Spermatozoen in ein Ei eintreten. Mehrere Spermakerne können mit dem Eikern verschmelzen und einen Triaster, einen Tetraster, oder eine unregelmäßigere Form einer Kernteilungsfigur hervorbringen.

4) In manchen Fällen furchen sich komprimierte, befruchtete Eier in anscheinend normaler Weise bis zum Blastulastadium; aber kein einziges entwickelt sich über dieses Stadium hinaus. Die Abnormität der Entwicklung wird gewöhnlich bei der ersten Furche offenbar und häufig teilen sich Eier auf einmal in drei, vier oder mehr Zellen. Stets erleidet die Teilung großen Aufschub.

5) Die Abnormitäten, welche durch Druckbehandlung der Eier hervorgebracht werden, beruhen teilweise auf der Formänderung des Eies und teilweise auf der Gegenwart einer abnormen Chromatinmenge.

6) Mängel der Sauerstoffzufuhr während dieser Kompression der Eier führt nicht zu Entwicklungsabnormitäten. Reife Eier in sauerstoffarmem Wasser zeigen die Reifungserscheinungen in normaler Weise und entwickeln sich, falls sie befruchtet sind, zu normalen Embryonen. Immerhin finden diese Prozesse viel langsamer statt als in Eiern, die sich normalerweise in Seewasser entwickeln.

Literature.

1) Boveri, M., Über Mitosen bei einseitiger Chromosomenbindung. Jen. Zeitschr. f. Naturwiss. Bd. XXXVII. 1903.

2) Boveri, Th., Zur Physiologie der Kern- und Zellteilung. Sitzungsber. d. Phys.-Med. Gesellsch. zu Würzburg. 1896.

3) Boveri, Th., Zellenstudien. V. Über die Abhängigkeit der Kerngröße und Zellenzahl der Seeigellarven von der Chromosomenzahl der Ausgangszellen. Jena 1905.
4) von Erlanger, F. R., Zur Kenntnis der Zell- und Kernteilung. II. Über die Befruchtung u. erste Teilung des Seeigeleies. Biol. Centralbl. Bd. XVIII. 1898.
5) Hartmann, M., Studien am tierischen Ei. I. Ovarialei und Eireifung von Asterias glacialis. Zool. Jahrb., Abt. f. Anat. u. Ontog. Bd. XV. 1902.
6) Hertwig, O. und R., Über den Befruchtungs- und Teilungsvorgang des tierischen Eies unter dem Einfluß äußerer Agentien. Jena 1887.
7) Hertwig, R, Über die Entwicklung des unbefruchteten Seeigeleies. Festschrift f. Gegenbaur. Bd. II. 1896.
8) Lefevre, G., Artificial Parthenogenesis in Thalassema mellita. Science. Vol. 21. 1905.
9) Loeb, J., and Lewis, W. H., On the Prolongation of the Life of the Unfertilized Egg of the Sea-Urchin by Potassium Cyanide. Amer. Journ. of Phys. Vol. VI. 1902.
10) Mathews, A. P., Artificial Parthenogenesis Produced by Mechanical Agitation. Amer. Journ. of Phys. Vol. VI. 1901.
11) Morgan, T. H., A Study of Variation in Cleavage. Archiv f. Entw.-Mech. Bd. II. 1895.
12) —— The Action of Salt Solutions on the Unfertilized and Fertilized Eggs of Arbacia and of other Animals. Archiv f. Entw.-Mech. Bd. VIII. 1899.
13) Wassilieff, A., Über künstliche Parthenogenesis des Seeigeleies. Biol. Centralbl. Bd. XXII. 1902.
14) Wilson, E. B., Experimental Studies in Cytology. I A Cytological Study of Artificial Parthenogenesis in Sea-Urchin Eggs. Archiv f. Entw.-Mech. Bd. XII. 1901.
15) —— Experimental Studies in Cytology. II. Some Phenomena of Fertilization and Cell-division in Etherized Eggs. Archiv f. Entw.-Mech. Bd. XIII. 1901.
16) Wilson, E. B., and Mathews, A. P., Maturation, Fertilzation, and Polarity in the Echinoderm Egg. New Light on the »Quadrille of the Centres«. Journ. Morph. Vol. X. 1895.
17) Ziegler, H. E., Experimentelle Studien über die Zellteilung. Archiv f. Entw.-Mech. Bd. VI. 1898.

Explanation of Plates.

Plates I and II.

All figures were drawn with the aid of a camera lucida under a Zeiss apoc. obj. 1,5 mm, oc. 4.

Fig. 1. Part of a section of an unfertilized egg that was under pressure for one and one-half hours, then removed from compression and killed one and one-quarter hours later.

Fig. 2. Part of a section of an egg from the same lot as that from which Fig. 1 was drawn.

Fig. 3. Part of a section of an egg from the same lot as that from which Figs. 1 and 2 were drawn.

Fig. 4. Part of a section of an unfertilized egg which was killed after being compressed for one and one-half hours.

Fig. 5. Part of a section of an unfertilized egg which had been compressed for one hour and forty-five minutes when killed.

Fig. 6. Part of a section of an egg from the same lot as that from which Fig. 5 was drawn.

Fig. 7. Part of a section of a fertilized egg which had been compressed for two hours when killed.

Fig. 8. Polar spindle in an unfertilized egg which was fixed after being compressed for one hour and ten minutes.

Fig. 9. Part of a section of a fertilized egg compressed for one and one-half hours and then killed one hour and fifteen minutes later.

Fig. 10. Part of a section of an egg from the same lot as that from which Fig. 9 was drawn.

Fig. 11. A polar spindle in a fertilized egg which had been compressed for two hours and fifteen minutes when killed.

Fig. 12. Part of a section of a fertilized egg that was killed after being compressed for one hour and twenty-five minutes.

Fig. 13. Part of a section of an egg from the same lot as that from which Fig. 12 was drawn.

Fig. 14. Part of a section of a fertilized egg compressed for two hours before fixation.

Fig. 15. Part of a section of an unfertilized egg compressed for one and one-half hours and then killed.

Fig. 16. Egg-nucleus in an unfertilized egg which had been under pressure for two and one-half hours before fixation.

Fig. 17. Part of a section of a fertilized egg which had been compressed for one and one-half hours, then removed from compression and fixed one hour and twenty-five minutes later.

Fig. 18. Polar spindle in a fertilized egg that had been compressed for one hour and forty-five minutes before fixation.

Fig. 19. Part of a section of a polyspermic egg fixed after compression for three and one-half hours. *E.G* egg-nucleus.

Fig. 20. Segmentation-spindle and pronuclei in a fertilized egg that was killed after being compressed for three and one-half hours.

Fig. 21. Segmentation-spindle in a fertilized egg that had been compressed for one hour and fifteen minutes and then fixed one hour and twenty minutes later.

Fig. 22. Section of a fertilized egg which was under pressure for one and one-half hours and then fixed two hours later.

Fig. 23. Part of a section of a polyspermic egg from the same lot as that from which Fig. 22 was drawn.

Fig. 24. Section of a polyspermic egg compressed for four hours before fixation.

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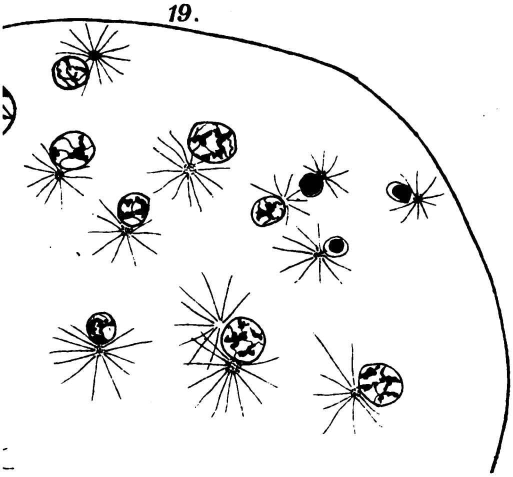

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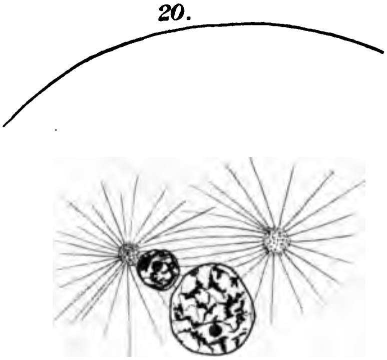

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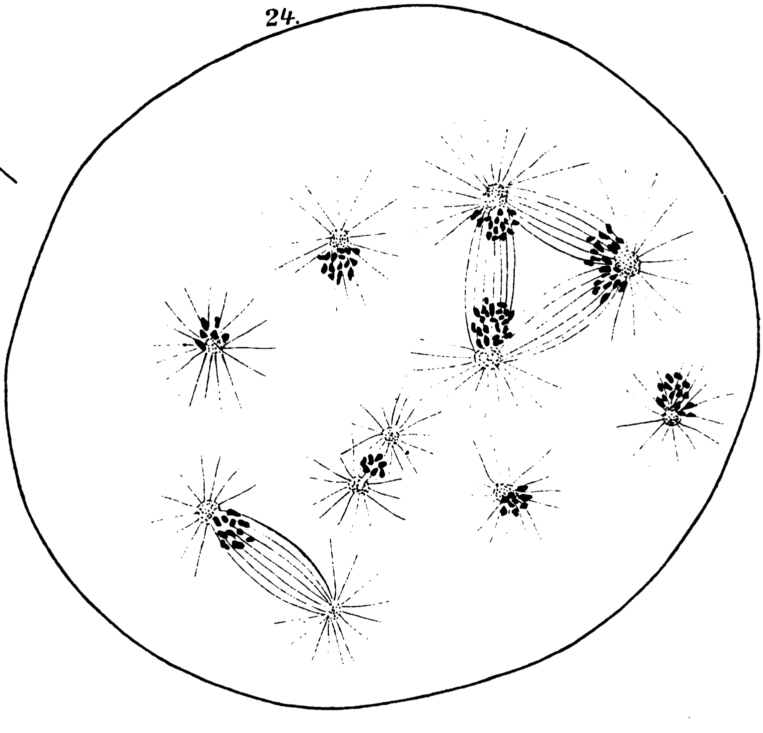

ann in Leipzig.

Lith. Anst. Julius Klinkha

PLANARIA MORGANI n. sp.

BY N. M. STEVENS AND A. M. BORING.

Size.—Length 10–12 mm., width in the region of the pharynx 1.6 mm., at the level of the eyes 1 mm.

Form.—Head wedge-shaped, no auricular appendages, posterior end obtuse, thickness slight compared with *Planaria maculata* or *P. simplicissima* (Plate I, figs. 1 and 2).

Color.—White and translucent; digestive tract often colored green, yellow, brown, or red by food recently ingested.

Eyes.—Two, crescent-shaped, situated far back and near together (Pl. I, figs. 1–4).

Nervous System.—Cephalic ganglia large and distinct, connected by a broad commissure, lateral nerve cords as in *P. maculata*, two prominent nerves extending forward from below the eyes (Plate I, fig. 4).

Pharynx.—Single, much elongated, centrally located in forms with functional sexual organs, posterior to the center in forms which are reproducing asexually (Plate I, figs. 1 and 2). Digestive tract as in the typical triclads with little anastomosis of branches (fig. 3).

Reproductive Organs.—Ovaries two, situated ventrally between the anterior axial division of the gut and its first lateral branches, sometimes lobed, and often extending nearly to the dorsal epidermis (Plate I, fig. 4). Oviducts following a spiral course dorsal and slightly lateral to the nerve cords, back to the region of the atrial organs, where they converge and unite into a short common oviduct which enters the atrium dorsal to the opening of the uterus stalk, instead of entering a vagina as in *P. maculata* (Curtis, '02). Spermatozoa are often found massed at the anterior end of the oviducts. Yolk glands lie between all of the main branches of the gut dorsal to the testes. Testes many, irregular in form and variable in size, scattered between the branches of the gut, for the most part ventral and median to the nerve cords, and extending from the region of the ovaries to the posterior end of the pharynx (fewer and larger than in *P. maculata*). Distinct seminal vesicles extending posteriorly from the last pair of testes (*v.s.*, Plate I, figs. 5 and 6). Vasa deferentia broad, short and only slightly coiled, entering the anterior enlargement of the lumen of the penis nearly at right angles. Penis short and broad, nearly filling the atrium (similar to that of *P. maculata*). Uterus large, dorsal, extending forward nearly to the pharynx chamber; stalk of the uterus passing to the left side, dorsal to the vasa deferentia and oviducts, and entering the atrium laterally and ventral to the entrance of the common oviduct (Plate I, figs. 5 and 6).

Reproduction by Fission.—Like *Planaria maculata,* this species reproduces both sexually and asexually. Indications that fission had occurred were observed in May, 1901–1905. In May, 1905, while all the individuals in one branch of a certain stream were sexually mature and depositing egg-capsules, those in another branch of the same stream were found in all stages of fission and regeneration (Plate I, figs. 7–10). During the last week of May and first week of June a few individuals showed regenerating atrial organs. The same difference, as to sexual maturity and fission, was observed in collections from the two branches of the stream in October, and at the date of writing (February 7th) there are no sexually mature worms among the thirty to forty specimens which were reproducing by fission when brought into the laboratory in October. About half of the number have divided in the aquarium. Fission occurs at a point nearer the pharynx than in *P. maculata* (fig. 7).

Regeneration.—Both anterior and posterior regeneration at all levels proceeds much as in *P. simplicissima,* but both regeneration and form regulation are more rapid.

Habitat.—*Planaria morgani* is found on the under side of stones and dead leaves in a small stream, one branch of which flows through the Bryn Mawr College campus. The authors have not found it elsewhere.

EXPLANATION OF PLATE I.

Fig. 1. Freehand sketch of living planarian, magnified five times. Atrial organs present (*a*). Limits of the digestive tract indicated by dotted outline.

Fig. 2. Same of an individual without atrial organs.

Fig. 3. Camera drawing of whole mount, showing pharynx and branches of the digestive tract. Magnification 10 diameters.

Fig. 4. Reconstruction from camera drawings of frontal sections, showing cerebral ganglia, lateral nerve cords, cephalic nerves, eyes, and ovaries. Mag. 35 d.

Fig. 5. Reconstruction of reproductive system from a series of frontal sections, dorsal aspect. *p.* = pharynx. *t.* = testis. *od.* = oviduct. *v.s.* = vesicula seminalis. *u.* = uterus. *p.l.* = penis lumen. *pe.* = penis. *a.* = atrium. *c.o.* = common oviduct. *r.* = reproductive pore. *u.s.* = uterus stalk. Mag. 10 d.

Fig. 6. Similar reconstruction from sagittal sections. Left-hand aspect. Oviduct and vas deferens of right side omitted. Lettering as above. Mag. 10 d.

Fig. 7. Freehand sketch of the anterior piece of a living specimen soon after fission. Mag. 5 d.

Fig. 8. Similar drawing from a larger anterior piece several days after fission, showing considerable regeneration.

Fig. 9. Posterior piece soon after fission, showing the two separate branches of the digestive tract. Mag. 10 d.

Fig. 10. Similar posterior piece after several days' regeneration (probably about a week). Branches of digestive tract united and new pharynx and eyes formed. Mag. 10 d.

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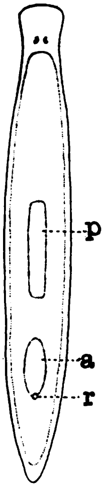

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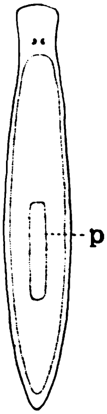

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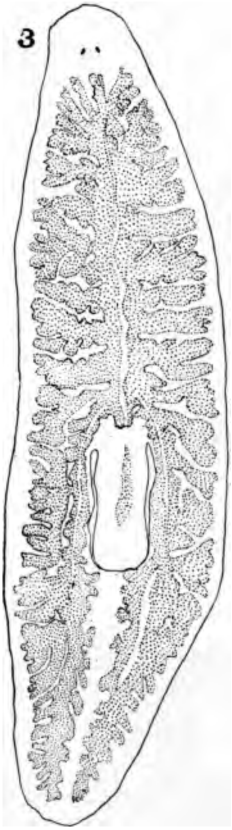

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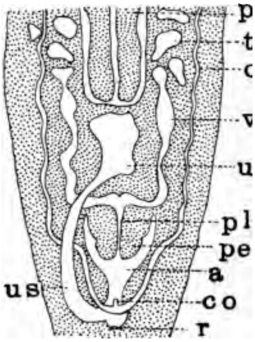

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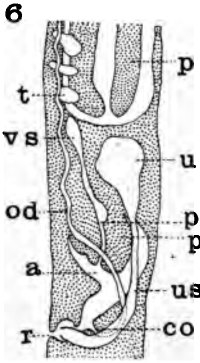

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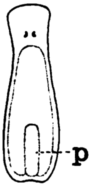

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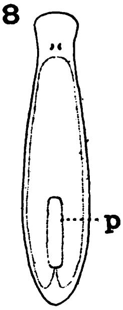

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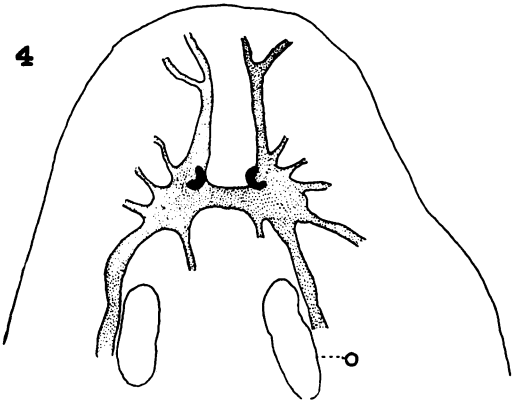

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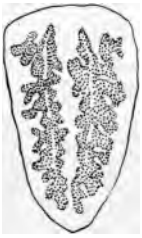

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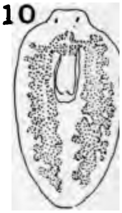

STEVENS AND BORING. PLANARIA MORGANI N. SP.

STUDIES ON THE GERM CELLS OF APHIDS

BY N. M. STEVENS

WASHINGTON, D. C.
Published by the Carnegie Institution of Washington
May, 1906

CARNEGIE INSTITUTION OF WASHINGTON

PUBLICATION NO. 51.

FROM THE PRESS OF
THE WILKENS-SHEIRY PRINTING CO.
WASHINGTON, D. C.

STUDIES ON THE GERM CELLS OF APHIDS.

By N. M. Stevens.

INTRODUCTION.

My previous report on the germ cells of aphids was based on a study of the parthenogenetic and winter eggs of *Aphis rosae*, the brown rose aphid, and of the spermatogenesis of *Aphis oenotherae*, found on the inflorescence of *Oenothera biennis*.

During the past summer and autumn material from more than twenty species has been collected and examined. In some cases it has been possible to study all of the forms of one species, in other cases only the parthenogenetic eggs, only the winter eggs, or only the spermatogenesis.

It has become evident in the course of the work that little dependence is to be placed on the present classification of aphids, and that a reclassification based on the cytology of the germ cells may be necessary. For example, it appears that at least three distinct species are usually included under the name *Aphis rosae*. No attempt will, therefore, be made in this paper to give the Latin names of the species studied, but they will be designated by the names of their host plants, and identified by brief description where it seems necessary.

This comparative study was undertaken in the hope of throwing more light on the question of sex determination, and the descriptive part of the paper will be followed by a further discussion of the bearing of the facts recorded on that problem.

METHODS.

The methods used were for the most part the same as in the previous work—fixation with Gilson's "acetic alcohol with sublimate" (Lee, sixth edition, p. 65) and staining by Heidenhain's iron-hæmatoxylin method. Some material was fixed in Flemming's fluid, Hermann's platino aceto-osmic, and Gilson's mercuro-nitric, but such fixation brought out no new points in structure, and was, in general, less satisfactory than fixation with Gilson's acetic alcohol-sublimate formula. The latter fluid was used at first on account of its great penetrating power, and because aphids will not sink in any water mixture; but by opening the insects with needles in the fluid any method of fixation may be used.

Material from different species, though fixed in the same way, takes the stain so differently that it has often been found necessary to restain several times to get just the right differentiation for counting and drawing the chromosomes accurately. Schneider's aceto-carmine proved to be useful in determining the character of new male material.

RESULTS OF INVESTIGATION.

The Rose Aphids.

On the roses of this section, both out of doors and in the greenhouse, one commonly finds a brown and a green aphid ; and on the various hardy roses there appear in autumn winged mothers of another species with large broods of white or yellowish winter-egg layers, and a little later the winged males are present. The host from which this latter species migrates to the rose for sexual reproduction has not yet been discovered, and the parthenogenetic forms have, therefore, not been studied ; it is, however, hoped that these may be obtained in the spring from eggs laid on isolated rose plants in the greenhouse.

In my last year's paper (Stevens, '05) the behavior of the female germ cells, both parthenogenetic and sexual, of the brown rose aphid was quite fully described ('05, pls. I–III, figs. 1–27), but males were not found. This year some males were obtained, but they were very few compared with the number of sexual females, and for some reason the material did not fix so well as that from most of the other species. A few good drawings of the first spermatocyte were made from aceto-carmine preparations of the testes taken from specimens collected on November 13 (pl. I, figs. 1–3). The metaphase of the second spermatocyte, from a section, is shown in figure 4. Figure 5, the metaphase of a segmentation spindle, and figure 6, the maturation mitosis of the parthenogenetic egg, confirm the results recorded in my earlier paper, showing the double series of maternal and paternal chromosomes in the parthenogenetic generation. No favorable maturation stages of the winter eggs were obtained. The scarcity of males was accounted for by the discovery of male and female embryos in the same individual, but only one or two males to many females. In this species the parthenogenetic females and the males are a reddish brown, while the sexual females are red and the eggs when first laid are green. The males are winged, the sexual females and the mothers of the sexual generations apterous.

The green rose aphid was studied last year only in the parthenogenetic form and the winter egg, and as there was some doubt as to the number of chromosomes, it did not appear in the published results. As in the case of the brown rose aphid, males are scarce, but some drawings were obtained from aceto-carmine preparations and from a few good sections. The number of chromosomes, seven in the spermatocyte (figs. 7–9, 11, 13, 14), shows this to be a distinct species, as had been indicated by experiments with the two kinds in the greenhouse. All of the forms of this aphid are green, and the freshly laid eggs are colorless.

Figures 7 to 9, drawn from aceto-carmine preparations on November 14, show the equatorial plate of the first spermatocyte. The amount of chromatin may not vary greatly from that in the corresponding cells of the brown aphid, but it is differently distributed. Figure 10 is a prophase of the first spermatocyte mitosis showing the side to side pairing of homologous chromosomes. Figure 11 is a metaphase of the first spermatocyte from a section, and figure 12 a late anaphase showing the lagging pair of chromosomes—characteristic of the first spermatocyte spindle in all of the species studied. Figures 13 and 14 are equatorial plates of the second spermatocyte, showing the same number and proportionate size of chromosomes as in the first spermatocyte. In figure 15 is shown the double series of 14 chromosomes in a two-cell stage of the parthenogenetic egg. This is also seen in figures 16*a* and 16*b*, two sections of the germinal vesicle of a parthenogenetic egg nearly ready for maturation. At this stage, as also in the prophase of segmentation mitoses, the chromosomes are larger and more irregular in outline than in the equatorial plate of the spindle.

The third, or migratory rose aphid, has so far yielded no results except from the winter eggs, which have been laid in considerable numbers on isolated rose bushes in the greenhouse. Only one polar spindle was found and that was cut diagonally. The nine chromosomes are shown in figures 17*a* and 17*b*; these, however, together with figures 18 and 19, equatorial plates of segmentation spindles of the winter egg, show the number and size relations of the chromosomes. The two broader and much longer chromosomes are without doubt homologous paternal and maternal elements, and the sixteen shorter ones can easily be paired.

Thus it appears that these three species of rose aphids are as distinctly separated by differences in number, form, and size of chromosomes (figs. 5, 15, 18) as they are by differences in external characteristics of form, size, and color.

The Willow Aphids.

In some material collected by Prof. Morgan from willow trees at Saranac, New York, early in September, were found a few young males and mothers of the sexual generation. Figures 20*a* and 20*b* are two sections of a first spermatocyte before the pairing of the chromosomes, each section containing one complete series of five. Figure 21 is a similar stage showing the two largest chromosomes and five of the smaller ones. Figure 22 shows two pairs of homologous chromosomes from a slightly later stage than that of figures 20 and 21. Figure 23 is the metaphase of the first spermatocyte, and figure 24 shows daughter plates of the anaphase. In figure 25 is seen a pair of second spermatocytes immediately after the formation of the nuclear membrane. The second division was not found, but a drawing of the young spermatid is given in figure 26. The number of chromosomes here is the same as in the brown rose aphid, but the proportionate size is somewhat different. There is more difference in size between the largest and the second in size, and the two smallest are proportionately larger than the corresponding ones in the rose aphid. The mothers of the sexual generations produce offspring of both sexes.

Much to my surprise, material collected on June 29 at Harpswell, Maine, contained sexual forms. These aphids were taken from the hairy, reddish young shoots of one of the dwarf willows, and they could not have been many generations away from the winter egg, for it was still early spring on the coast of Maine. There were a few young males and females, and the apterous parthenogenetic mothers contained embryos in the following combinations :

1. All parthenogetic.
2. All sexual female.
3. Parthenogenetic and sexual female.
4. Parthenogenetic male.
5. Parthenogenetic male and female.

Kyber (1815) mentions finding sexual forms on the willow the last of June, but attributes their presence to the change in sap conditions of the host at the close of the growing season, while in this case they were found on young growing shoots.

The polar spindle of the parthenogenetic egg in metaphase is shown in figure 27, and prophases of segmentation spindles in figures 28 and 29. Six chromosomes, which evidently form three pairs, are present. Figure 30 shows resting cells of a segmenting parthenogenetic egg. The conspicuous nucleolus with its several vacuoles divides into two as a prophase of nuclear and cell divisions. Figures 31 and 32 are first spermatocytes in metaphase and figures 33 and 34 in anaphase;

here the smaller chromosome appears always to be the one to lag behind. Figure 35 shows the partial resting stage between the two spermatocyte mitoses. No second spermatocytes were found in division. The same side to side pairing of chromosomes as in the other species was observed.

This species has the smallest number of chromosomes of any of those studied, and it apparently has the smallest amount of chromatin, if we may judge by comparison of the metaphase of the first spermatocyte in the various species.

The Oenothera Aphids.

As the spermatogenesis of *Aphis oenotherae* was fully described last year (Stevens '05, pl. IV, figs. 28-42), only two new figures of the first and second spermatocyte will be introduced for comparison with other species having the same number of chromosomes (figs. 36 and 37.) The size relation resembles quite closely that of the brown rose aphid, with which it was compared in my former paper, but the largest chromosome is usually more elongated and the difference between the two largest is greater. (Compare figs. 3 and 36.) In external appearance *Aphis oenotherae* is very different from *Aphis rosae*. The females are a bright red and the males and their apterous mothers green. This aphid was not found on the young plants at Bryn Mawr in spring, or on the Oenotheras at Harpswell, Maine, or Woods Hole, Massachusetts, during the summer. It may therefore have a second host,* from which it migrates to *Oenothera biennis* in the late summer or autumn.

Another smaller, green aphid was found in abundance on the foliage of the Oenotheras at Harpswell, Woods Hole, and Bryn Mawr. The parthenogenetic form of this aphid is light green, the winter egg-layers darker with a blackish longitudinal stripe on the back, and the apterous males brownish and very small. This was the only species in which apterous males were observed. The equatorial plate of the maturation spindles of the parthenogenetic egg, with eight chromosomes, is shown in figure 38, the metaphase of the first spermatocyte in figures 39 and 40, the anaphase in figures 41 and 42, and a spermatid in figure 43.

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* *Aphis oenotherae* has recently hatched in considerable numbers on rosettes of *Oenothera biennis*, where the eggs were laid in the greenhouse in October. The egg layers were red, but their offspring are both red and green, and individuals of either color, isolated, have produced offspring of the same color in the second and third generations. If they continue to breed true to color an attempt will be made to keep several lines of each color isolated until the sexual generation appears in the autumn.

The Milkweed Aphids.

On the milkweeds at Woods Hole, only one kind of aphid was found, a very dark colored one—nearly black—which lived in herds attended by ants. Sexual forms were collected on September 27. The metaphase of the maturation mitosis of the parthenogenetic egg is shown in figure 44, and that of a spindle of the 4-cell stage in figure 45. The prophase of a spermatogonial mitosis appears in figure 46, exhibiting the same double series of chromosomes as in the parthenogenetic egg. The metaphase of the first spermatocyte is shown in figure 47, and daughter plates of the anaphase in figure 48. The lagging chromosome in the anaphase (fig. 49) is here the smallest of the four. Figures 50 and 51 show different views of the anaphase of the second spermatocyte. The number of chromosomes is the same as in the second Oenothera aphid, but the difference in form and size is evident. (Compare figs. 38 and 44, 39 and 47.)

On the milkweeds at Bryn Mawr two species were found in abundance, one pale (white with some brown spots), and the other a brilliant orange. The latter remained parthenogenetic until the plants were destroyed by frosts, no sexual forms appearing. A few drawings were made from the parthenogenetic egg. Figures 52 and 53 are the metaphase of the maturation spindle, with two chromosomes conspicuously larger than the other six. Figure 54 is a similar stage, but the two largest chromosomes are evidently united. Two equatorial plates of this kind were found in embryos of one mother, while the usual number—eight—appeared in segmentation stages of eggs in the same embryos. In this species the chromosomes become distinct and take stains well from the moment when the growth stage of the oöcyte begins. A young oöcyte, just leaving the ovary, is shown in figure 55. A large plasmosome (*p*) and six of the chromosomes appear in this section. A prophase of mitosis in a 16-cell stage is shown in figure 56, and a section of a 4-cell stage, showing the polar body (*pb*) and the metaphase of three spindles, in figure 57.

In the spermatocytes of the pale milkweed aphid there are seven chromosomes, always grouped in a characteristic way (figs. 58 and 59). The smallest chromosome is closely associated with the next in size and the two occupy the center of the plate. The arrangement suggested the conditions in some of the Hemiptera heteroptera, but careful examination of the anaphase of both maturation spindles showed that these two chromosomes divide like the others, both, however, lagging behind the others (figs. 60 and 61). Figure 62 is a daughter plate of the first spermatocyte. Figures 63 and 64 are slightly different stages in the pairing of the chromosomes as a prophase of the first maturation mitosis. In the second spermatocyte the two smallest chromosomes are as widely separated as any other two (fig. 65). One individual in this material had nine instead of seven chromosomes in the spermatocytes, the two smallest closely associated in the center of the plate and seven in the outer circle. This was probably a stray specimen of another species, perhaps closely related, for here the arrangement of the chromosomes seems to be as characteristic of the species as their form and size. One day's collection of this material contained several individuals which, on dissecting, showed both embryos and winter eggs from the same ovaries, thus confirming what I found once among my rose aphids in the greenhouse; but the fact that I collected and examined a great deal of the same kind of material from the same locality on the following days, and found no more cases of this kind, shows it to be of rare occurrence.

In the black and the orange milkweed aphids we have two conspicuously different aphids with chromosomes of the same number and not markedly different size relations. A similar looking aphid to the black milkweed aphid was found on the garden nasturtium, and here the number and size of the chromosomes is near enough the same to make it probable that they are the same species. Figures 66, 67, and 68 show the prophase and metaphase of the first spermatocyte of the nasturtium aphid.

The Oak Aphids.

On the red oak were found some peculiarly marked red and yellow aphids, the males having spotted wings. These have seven chromosomes in the spermatocytes (fig. 69). Figure 70 is a daughter plate of a first spermatocyte, figure 71 an equatorial plate, and figure 72 a daughter plate of a second spermatocyte. One specimen in this material had eight chromosomes, the arrangement being similar to that in the pale milkweed aphids, but the number different (figs. 73 and 74).

On another species of oak was found a white aphid with seven chromosomes, varying considerably in size from those of the red oak aphid. First spermatocytes are shown in figures 75 to 78. These figures show no uniformity of grouping. In fact, the only point of uniformity so far discovered in any species is the close association of the same two chromosomes in the first spermatocytes of the pale milkweed aphids and the one stray red oak aphid (figs. 58 and 73).

The Goldenrod Aphids.

The goldenrods, besides serving as hosts for several beetles, offer to the insect collector at least two distinct species of aphids. On the beach goldenrod are large numbers of dark brown aphids with green males. The sexual forms were found at Woods Hole on September 27. The polar body (*pb*) and the segmentation nucleus of the parthenogenetic egg are shown in figures 79 *a* and 79 *b*, the metaphase of the first spermatocyte in figures 80 and 81, one daughter plate of a late anaphase in figure 82, the metaphase of the second spermatocyte in figure 83, and one of the daughter plates of an anaphase in figure 84. In this species four of the chromosomes are of nearly the same form and size, the other two being very much smaller and one slightly smaller than the other. This was the first aphid in which both male and female embryos were found in sections of the same individual. This was quite unexpected, as in the forms studied last year, particularly the Oenothera aphid and the migratory rose aphid, the male and female sexual generations seemed to be entirely separate, the former from apterous, the latter from winged mothers.

The tall goldenrod, *Solidago altissima*, is the host of quite a different aphid with the same number of chromosomes. The ordinary parthenogenetic individuals and the sexual females are green, the males red, and the mothers of the sexual generation either green or red, individuals of both colors giving rise to both male and female progeny. Figures 85 and 86 are prophases of the first spermatocyte mitosis, figure 85 before the pairing of the chromosome and figure 86 during that process. Figure 87 is the metaphase and figure 88 an early anaphase of the first spermatocyte from material fixed with Gilson's fluid, figures 89 and 90 from Flemming material. Figures 91 and 92 are the metaphase of the second spermatocyte. The chromosomes, though the same in number, will be seen to differ considerably from those of the brown goldenrod aphid in both form and size.

The Clover, Birch, and Beech Aphids.

On the paper birch were found the sexual forms of a brown aphid. Most of the males were too old to be of use and there were no parthenogenetic forms when they were discovered late in October. Only one drawing of the first spermatocyte, showing nine chromosomes, was made from an aceto-carmine preparation (fig. 93). Only a few young males of the clover aphid were obtained. The testes of these showed a characteristic metaphase of the first spermatocyte containing one large chromosome surrounded by a circle of seven nearly equal in size (fig. 94). Figures 95 and 96 are prophases of the first maturation mitosis.

The first and second spermatocytes of the woolly beech aphid have such equatorial plates as are seen in figures 97, 98, and 99. The anaphase (figs. 100 and 101) is rather interesting, as here the lagging chromosome is evidently the largest.

The Star Cucumber Aphid.

This aphid was discovered quite late, just before the frost killed the host plant, and only the sexual generation with the mothers of the males and females was present. The males were winged and red, the females pale green, and the mothers of the sexual generation were, some red, others bright green. Here again the same mother gives rise to both sexes.

Some of this material was fixed in Hermann's fluid, and figures 102 to 105 were taken from the Hermann material. This fixation was no better for the mitotic phases than that obtained with the Gilson formula, but the nuclei of the young spermatocytes showed more definite structure (figs. 102 and 103). The plasmosome did not take the hæmatoxylin stain, but remained a yellowish gray, while the chromatin appeared in a variously tangled and wound feathery spireme. Figure 106 also shows the metaphase of the first spermatocyte and figure 107 an early anaphase. Figure 104 is a prophase showing the paired condition of one of the longer chromosomes, and figures 108 and 109 are pairs of daughter plates from the second spermatocyte. In this aphid we have a marked difference in the size relation of the chromosomes from that in the other species that have the same number. (Compare figs. 3, 23, 36, 105 or 106).

The Maple Aphid.

This is a pale aphid—white with some brown markings. Its chief peculiarity consists in the large number of chromosomes—sixteen in the spermatocytes. As shown in figure 110, one chromosome is always much larger than the rest, and the other fifteen vary only slightly in size. There are often two lagging chromosomes (fig. 111) in the anaphase of the first spermatocyte, and one daughter plate is usually concentrated before the other, giving the unsymmetrical appearance of figure 112. In figure 113, a prophase of the first spermatocyte mitosis, paired chromosomes are shown. The parthenogenetic forms were not examined.

The Pea Aphid.

The pea aphid, or pea louse, as it is commonly called, shows a very neatly paired double series of eight chromosomes in the parthenogenetic generations. Figure 114 is from a maturation spindle and figures 115 and 116 from a 32-cell stage of the parthenogenetic egg. The sexual forms were not available for study.

The Goumi Aphid.

On the shrub known as the goumi is found an interesting aphid of which I discovered the sexual forms too late to get anything of the spermatogenesis or to find the parthenogenetic forms. The males were small and nearly black; the females of three distinct colors—green, red, and yellow—but all were marked alike with a darker stripe on the back. When brought into the laboratory they laid their orange-colored eggs in abundance on a white cloth tied over the jar in which they were confined to prevent their traveling all over the laboratory.

Figures 117 and 118 show polar bodies containing five chromosomes, and figure 119 the male and female pronuclei in contact, the female nucleus being the larger and containing two plasmosomes (*p*).

Figures 120 *a* and 120 *b* show a prophase of mitosis from an 8-cell stage. Such stages are of course not so good for comparison of the chromosomes as the equatorial plate, but the two series of homologous chromosomes are fairly well shown, considering that they do not all lie in the same plane or even exactly parallel with the surface of the section.

In many cases more stages might have been figured, but it did not seem advisable to multiply figures more than was necessary to show the characteristic appearance and behavior of the chromosomes of each species. Several drawings of the same stage have often been given to show the variations in arrangement of the chromosomes.

SUMMARY OF RESULTS.

1. Each species of aphids is characterized not only by a specific number of chromosomes, but by peculiarities in their form and size, and in some cases by a definite arrangement, as in the clover aphid, the pale milkweed aphids, and the second oak aphid described (figs. 58, 59, 73, 74, 94). Where the number of chromosomes is the same in two species, there is always some characteristic difference in form or size corresponding to the external differences in the species.

2. No evidence of hybridism has been observed where two or more species occur on the same host.

3. There is no evidence of any reduction in the number of chromosomes, or of more than one maturation mitosis in the parthenogenetic generations.

4. A double series of homologous paternal and maternal chromosomes runs through the parthenogenetic generations, and the homologous chromosomes are paired side to side in the first spermatocyte, and presumably in the oöcyte, of the sexual generation (figs. 10, 22, 63, 64, 68, 86, 104, 113).

5. The first spermatocyte mitosis is the reducing division, separating homologous chromosomes paired during the prophase.

6. There are no heterochromosomes of any kind, but the spermatids are all alike so far as number, form, and size of chromosomes is considered.

7. The same parthenogenetic individual may produce:
   (1) All parthenogenetic embryos.
   (2) Parthenogenetic embryos and winter eggs.
   (3) All sexual female embryos.
   (4) All male embryos.
   (5) Parthenogenetic and sexual female embryos.
   (6) Parthenogenetic and male embryos.
   (7) Parthenogenetic, male, and sexual female embryos.
   (8) Male and sexual female embryos.

GENERAL DISCUSSION.

The Relation of Species.

If we may suppose that all the species of aphids having the same number of chromosomes have come from the same ancestors, the mutations may be indicated by such differences in the form and size relations of the chromosomes as may be seen by comparing the first spermatocyte of the brown rose aphid, the Saranac willow aphid, the Oenothera aphid, No. I, and the star cucumber aphid, with five chromosomes (figs. 3, 23, 36, 105); or that of the black milkweed aphid, the Oenothera aphid No. II, and the nasturtium aphid with four chromosomes (figs. 39, 47, 66); or again, the metaphase of the maturation spindle of the parthenogenetic egg of the orange milkweed aphid and the pea aphid with eight chromosomes for the somatic number (figs. 52, 114). It is evident that mutations might as easily occur by a change involving the number of chromosomes as their form and size.

The three species having the two smallest chromosomes closely associated in the metaphase of the first spermatocyte (figs. 58 and 73), but differing in the number of chromosomes—seven, eight, and nine—were similarly marked and probably closely related.

The five species having five chromosomes for the reduced number were obviously different in form, size,and color, while of those having four chromosomes in the spermatocytes only the black milkweed aphid and the nasturtium aphid were nearly enough alike to suggest that they might be the same species, though one was found on milkweed at Woods Hole, Massachusetts, and the other on garden nasturtiums at Bryn Mawr, Pennsylvania.

The two oak aphids, where the number and size relations of the chromosomes are similar (figs. 69 and 76), were very unlike in appearance when collected, one being red and yellow, the other nearly white with some brown dots; but the alcoholic specimens, minus their coloring, were strikingly alike.

The two goldenrod aphids, with the same number of chromosomes but somewhat different form and size relations (figs. 80 and 87), are extremely unlike in appearance, one being a dark reddish brown with green males, the other green with bright red males.

This examination of a few out of the hundreds of species of aphids has indicated to me that an intelligent classification of these insects can be hoped for only when a careful study of the external characters and habits of the various species is combined with an equally careful study and comparison of their germ cells. My work can be regarded as at present the smallest beginning of such a study, and is merely suggestive of what may be necessary in the way of a combination of systematic with cytological methods.

Mendel's Law and the Individuality of the Chromosomes.

It would hardly be possible to find better material than the germ cells of the aphids to illustrate the individuality of the chromosomes, and the probable working of Mendel's Law of Heredity.

In every one of the twenty-four species examined some or all of the chromosomes possess characteristics which distinguish them from their fellows, and these peculiarities persist throughout all the generations. In every species where it has been possible to study and compare the germ cells of the parthenogenetic and sexual generations, the single series of the maturating sexual germ cells has been found to be exactly duplicated in the double series of the parthenogenetic egg, the segmenting winter egg, and the spermatocytes before reduction; and there seems to be no room for doubt that homologous maternal and paternal chromosomes are paired and then separated in maturation.

That the spermatids must be "pure" with regard to the paternal and maternal characters represented by the several pairs of chromosomes is indicated by the fact that in the spermatocyte pairing of the chromosomes does not occur until the prophase of the first maturation mitosis, and even then the homologous chromosomes are merely paired without close union. There is therefore little opportunity for mingling of the chromatin elements of any two paired chromosomes.

In such a case as that of the Harpswell willow aphid with only three chromosomes in the spermatocyte there must be extreme correlation of characters, as only six different combinations of maternal and paternal chromosomes would be possible in the mature eggs and the spermatozoa, while in the maple aphid with sixteen chromosomes, a large variety of different combinations of maternal and paternal characters is possible. Why there should be so marked a difference in number of chromosomes and in amount of chromatin in the several species is at present inexplicable. I thought at first that it might be possible to homologize the various series, and see where a single chromosome in one species might be equivalent to two or more in another, the amount of chromatin being approximately the same in all. A comparative glance at the plates is enough to show that such an attempt would be futile. The difference in amount of chromatin in the same stage of maturation—for example, the metaphase of the first spermatocyte, in material treated in exactly the same way—though not so great as the difference in number, is still very evident.

SEX DETERMINATION.

Though this study was undertaken with the hope that it, together with experimental work, might throw considerable light on the much discussed question of sex determination, it has yielded more abundant results along other lines.

The discovery of embryos and winter eggs in the same parthenogenetic individual has been confirmed, but shown to be of rare occurrence. However, the fact that this can occur is evidence that the parthenogenetic and winter eggs are different in method of development, rather than in their original constitution.

Several new points as to the relation of the sexual to the parthenogenetic generations have been gained (Summary of Results, No. 7). It will be seen at once that these relations are such as to prove conclusively that the changes in sex usually attributed to changes in external conditions are really a change from the parthenogenetic to the sexual mode of reproduction. This point has been fully discussed by Castle in his paper on the Heredity of Sex, page 190 ('03). It might still be maintained that different external conditions had caused some eggs to develop into male and others into female embryos in the same individual, had not embryos been repeatedly found of different sexes and of exactly the same size, and presumably of too near the same age to make it probable that the developing eggs had been given their sex impulse by different external conditions. Careful examination was made to see whether, in cases where embryos of both sexes occurred, all from one ovary were of the same sex, and also whether, if both sexes come from the same ovary, there is any definite order of succession in the oviducts. These are not easy questions to answer from sections which cut the embryos in various planes, but it was ascertained that both sexes occur in the same oviduct, and that embryos of different sexes may alternate or two or more successive embryos may be of the same sex.

From the results recently obtained in the study of the germ cells of other insects [McClung ('99-'05), Sutton ('02, '03), Wilson ('05) Stevens ('05)], it now seems probable that the sex character is represented in the chromatin in the germ cells and behaves like other Mendelian characters, as suggested by Castle ('03). Assuming that this is true, the spermatozoa must be dimorphic with respect to the sex character—one half of them containing the male character, the other half the female character—since the mothers of the males may produce both male and female offspring, and there is no reduction of chromosomes in the eggs which develop into male embryos.

It is evident that every fertilized aphid egg must contain the female sex character, since it invariably produces a parthenogenetic female. The possible combinations of parthenogenetic, sexual female, and male embryos cited in No. 7 of the Summary of Results, page 13, show that in all probability every parthenogenetic female is capable of producing sexual female progeny; in other words, the female character is dimorphic, if one may so describe it. It gives rise under some conditions to the development of a parthenogenetic female, under other conditions to a sexual female. Now it remains to be determined whether (1) each fertilized egg also contains the male character recessive (Castle, '03), or (2) whether only a part of the eggs contain the male and female characters as alternates, while the remainder have the female character in both the maternal and paternal series of chromosomes. The former condition—male and female characters in all eggs—would imply maturation of the winter eggs according to the law of chances and meeting in fertilization of only eggs and spermatozoa containing different sex characters (Castle, '03). The latter condition might be the result of such a differential maturation of the eggs that all should contain only the female sex character before fertilization. The sex possibilities of the fertilized egg would then be determined by the entrance either of a spermatozoön containing the male character or of one containing the female character. An egg fertilized by a spermatozoön containing the female character would then produce a line of aphids consisting only of parthenogenetic females and sexual females. An egg fertilized by a spermatozoön containing the male element would be capable of producing a succession of generations consisting of parthenogenetic females, sexual females and males. This is just what we find (Summary, No. 7), but the same conditions could occur if all the fertilized eggs contain both male and female characters.

The fact that in some species (Oenothera aphid No. I and the white rose aphid) the two sexes are entirely separate in the sexual generation, and that in the six species in which embryos of both sexes are found together there are always many individuals that contain only female embryos, suggested the possibility that there might be in each species entirely separate lines from the fertilized egg through the parthenogenetic generations to the sexual generation, some lines being capable of producing males, others not. The only way to test this point would be to isolate parthenogenetic individuals of a species, either directly from the fertilized egg or from somewhat widely separated hosts, and examine the sexual generations from each. If many individuals from some lines of descent should give only sexual females, while those from other lines gave either only males or both males and females, the natural conclusion would be that some fertilized eggs contain the male character, while others do not. If, on the other hand, both males and females should occur in all the lines of descent, we should feel reasonably sure that all the fertilized eggs contain both the male and female character.

Unfortunately there is no such visible dimorphism in the spermatids of the aphid as in some of the Hemiptera, Orthoptera, and Coleoptera to serve as direct evidence of sex-determining power, but if the sex characters are represented in the chromatin as Mendelian alternates there is abundant evidence that the spermatogonia must contain both characters and that the spermatozoa must be pure as to the two alternating characters.

As to which seems the more likely supposition (1) that only germ cells with opposite sex characters can unite to give a fertile egg, or (2) that all the winter eggs can be so maturated that only the female character remains, one proposition seems about as likely to be true as the other; and at present I see no third possibility, since there is no evidence that the female element is removed from the parthenogenetic eggs that produce males (Castle,'03) and the spermatozoa must therefore be of two kinds respecting the sex characters, just as in the case of any other two homologous maternal and paternal characters.*

Whatever evidence isolation experiments may give on this point ot the distribution of the male and female characters in the fertilized egg, we know that from one parthenogenetic egg can come an individual whose germ cells can produce (1) parthenogenetic, (2) sexual female, or (3) male offspring, and so far as we can see the indications are that the same influences that cause a change in the mode of reproduction from parthenogenetic to sexual produce in some species or in some individuals of a species a definite change in dominance of the sex characters (Oenothera aphid No. I and white rose aphid), while in other species (goldenrod and willow aphids) a more or less balanced state of dominance must be associated with the initiation of sexual reproduction and some unknown internal factor determine which sex character shall control the development of each egg. The whole question of sex determination will be seen to be far more complex in the case of the aphids than in insects where no parthenogenetic generations intervene between the fertilized egg and the production of males.

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* In the case of *Aphis oenotherae* it is possible that the germ cells of the red parthenogenetic individuals contain the female sex character in both the paternal and the maternal series of chromosomes, while the green individuals have the female sex character in the maternal series and the male in the paternal series, the green color being correlated with the male sex character and dominating over the maternal red. The former line would then give rise to sexual females and winter eggs containing only the female character; the latter, by a change in dominance of the sex character, would produce males containing both characters. Fertilization by the resulting two classes of spermatozoa would then perpetuate the two sexual lines.

In collecting aphids this year it was found that the sexual generation appeared in many species from two to three weeks earlier than in 1903 and 1904, though the weather continued warm much later than during the two preceding autumns. This seemed to be related to the earlier ripening up of the various host plants. The earlier fruiting and drying up was especially conspicuous in *Oenothera biennis* and some of the rose bushes. Sexual forms of both the brown and green rose aphid were found on young growing tips of these same bushes, but they may have been affected by the general sap conditions of the plant. Finding sexual forms among the willow aphids collected from young shoots at Harpswell, Maine, on June 29, suggested that in some species there may be definite cycles of generations not directly subject to external conditions, as Weismann maintains for the Daphnids ('76, '79). This species of aphids deserves further investigation.

One is also somewhat mystified by the fact that in some species there is a sudden and complete change from the parthenogenetic to the sexual mode of reproduction, while in others, as for example the brown and the green rose aphids, parthenogenetic generations continue to multiply until destroyed by the cold of winter. The complete change seems to be the rule in the majority of the species in this climate, and is much more easily explained than the mixed condition.

So far it has not been possible to produce the sexual generations by subjecting the aphids to artificial changes of conditions. This may be due to the fact that the brown and green rose aphids, which have been used for most of the experiments, may be the most unfavorable for such work, as they produce scattered sexual forms while continuing the parthenogenetic generations, indicating either that they are less sensitive to changes in environment or that they are less well adapted to this climate.

This is evidently a case where experimental work must be based on rather extensive observation and cytological research, and the results recorded in this paper give a far better basis for experiment than we had a year ago.

BRYN MAWR COLLEGE, *January 15, 1906.*

BIBLIOGRAPHY.

CASTLE, W. E.

'03. The heredity of sex. Bull. Mus. Comp. Zoöl. Harvard College, vol. 40, no.4.

KYBER, J. F.

'15. Einige Erfahrungen und Bemerkungen über Blattläuse. Germar's Magazin der Entomologie, t. 1–2c.

McCLUNG, C. E.

'99 A peculiar nuclear element in the male reproductive cells of insects. Zoöl. Bull., vol. 2.

'00. The spermatocyte divisions of the Acridiidæ. Kans. Univ. Quart., vol. 9, no. 1.

'01. Notes on the accessory chromosomes. Anat. Anz., bd. 20, nos. 8 and 9.

'02. The accessory chromosome—Sex-determinant? Biol. Bull., vol. 3, nos. 1 and 2.

'02a. The spermatocyte divisions of the Locustidæ. Kans. Univ. Quart., vol. 1, no. 8.

'05. The chromosome complex of orthopteran spermatocytes. Biol. Bull., vol. 9, no. 5.

STEVENS, N. M.

'05. A study of the germ cells of *Aphis rosae* and *Aphis oenotherae*. Journ. of Exp. Zoöl., vol. 2, no. 3.

'05a. Studies in spermatogenesis, with especial reference to the "accessory chromosome". Carnegie Inst. of Wash., pub. no. 36.

SUTTON, W. S.

'02. On the morphology of the chromosome group in *Brachystola magna*. Biol. Bull., vol. 4, no. 1.

'03. The chromosomes in heredity. Biol. Bull., vol. 4, no. 5.

WEISMANN, A.

'76–79. Beiträge zur Naturgeschichte der Daphnoiden. Leipzig.

WILSON, E. B.

'05. The behavior of the idiochromosomes in Hemiptera. Journ. of Exp. Zoöl., vol. 2, no. 3.

'05a. Studies on chromosomes. II. The paired microchromosomes, idiochromosomes, and heterotropic chromosomes in Hemiptera. Journ. of Exp. Zoöl., vol. 2, no. 4.

DESCRIPTION OF PLATES.

[The figures were all drawn with camera lucida, Zeiss oil-immersion 2 mm., oc. 12 and have been reduced one-third, giving a magnification of 1000 diameters.]

PLATE I.

Brown rose aphid.

FIGS. 1-3. First spermatocyte, metaphase, aceto-carmine preparation of November 13.
4. Second spermatocyte, metaphase.
5. Parthenogenetic egg, metaphase of segmentation spindle.
6. Parthenogenetic egg, metaphase of maturation spindle. The double series of chromosomes is numbered, here and in other figures, in order of size.

Green rose aphid.

7-9. First spermatocyte, metaphase, aceto-carmine preparation of November 14.
10. First spermatocyte, prophase, showing paired chromosomes.
11. First spermatocyte, metaphase, from section.
12. First spermatocyte, late anaphase.
13-14. Second spermatocyte, metaphase.
15. Two-cell stage of parthenogenetic egg, metaphase, showing the double series of 14 somatic chromosomes.
16 *a* and *b*. Parthenogenetic egg, prophase of maturation mitosis showing 14 chromosomes.

Migratory rose aphid.

17 *a* and *b*. Winter egg, first maturation mitosis, metaphase, 9 chromosomes.
18-19. Winter egg, metaphase of segmentation spindles, 18 chromosomes.

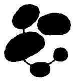
Saranac willow aphid.

20 *a* and *b*. First spermatocyte, prophase, showing double series of 10 chromosomes.
21. First spermatocyte, prophase.
22. Paired chromosomes from prophase of first spermatocyte.
23. First spermatocyte, metaphase.
24. First spermatocyte, anaphase.
25. A pair of second spermatocytes.
26. Young spermatid.

Harpswell willow aphid.

27. Parthenogenetic egg, metaphase of maturation spindle.
28-29. Parthenogenetic egg, prophases of segmentation mitosis.

STEVENS PLATE I.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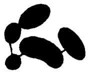
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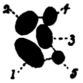
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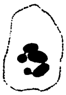
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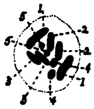
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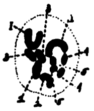
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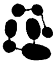
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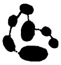
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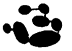
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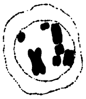
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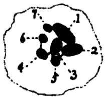
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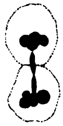
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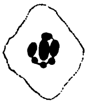
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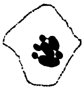
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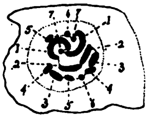
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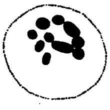
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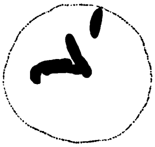
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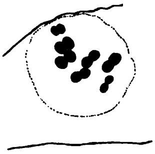
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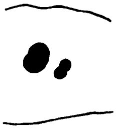
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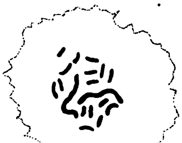
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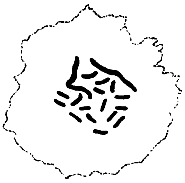
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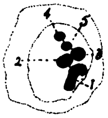
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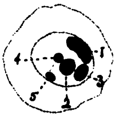
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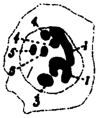
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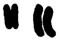
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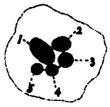
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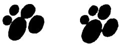
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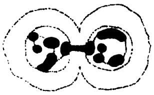
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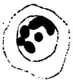
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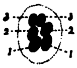
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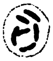
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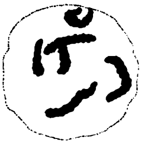
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PLATE II.

Harpswell willow aphid.

FIG. 30. Parthenogenetic egg, resting nuclei, showing nucleolus divided before mitosis.
31-32. First spermatocyte, metaphase.
33-34. First spermatocyte, anaphase.
35. Second spermatocyte, prophase.

Aphis oenotherae.

36. First spermatocyte, metaphase.
37. Second spermatocyte, metaphase.

Oenothera aphid, No. II.

38. Parthenogenetic egg, metaphase of maturation spindle.
39-40. First spermatocyte, metaphase.
41. First spermatocyte, early anaphase.
42. First spermatocyte, late anaphase.
43. Young spermatid.

Black milkweed aphid.

44. Parthenogenetic egg, metaphase of maturation spindle.
45. Parthenogenetic egg, 4-cell stage, metaphase of segmentation spindle.
46. Spermatogonium, prophase of mitosis.
47. First spermatocyte, metaphase.
48-49. First spermatocyte, anaphase.
50-51. Second spermatocyte, anaphase.

Orange milkweed aphid.

52-53. Parthenogenetic egg, metaphase of maturation spindle.
54. Parthenogenetic egg, metaphase of maturation spindle, showing the two largest chromosomes united (1+1).
55. Young oöcyte, showing plasmosome (*p*) and six of the chromosomes.
56, Parthenogenetic egg, 16-cell stage, prophase of mitosis.
57. Parthenogenetic egg, polar body (*p. b.*) and three segmentation spindles.

STEVENS

PLATE II.

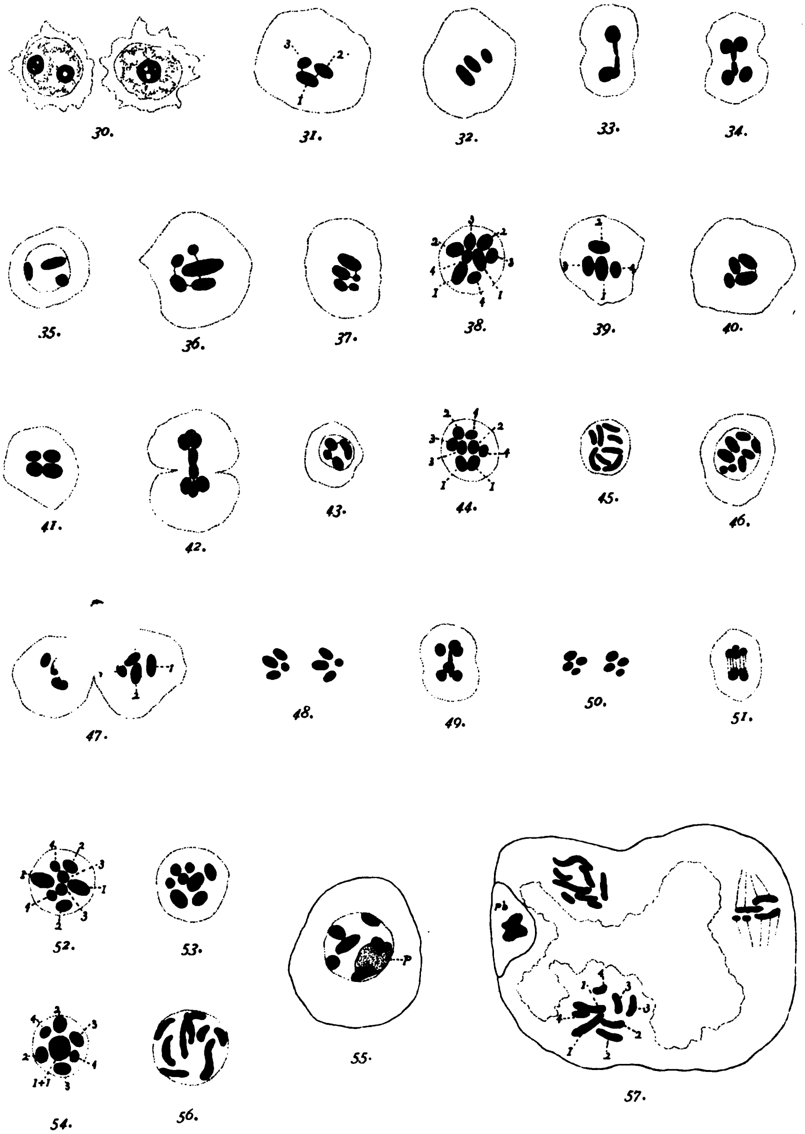

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PLATE III.

Pale milkweed aphid,

FIGS. 58-59. First spermatocyte, metaphase.
60-61. First spermatocyte, anaphase.
62. First spermatocyte, daughter plate.
63-64. First spermatocyte, prophase. showing paired chromosomes.
65. Second spermatocyte, metaphase.

Nasturtium aphid.

66. First spermatocyte, metaphase.
67-68. First spermatocyte, prophase.

Oak aphids.

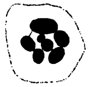
69. No. I, first spermatocyte, metaphase.
70. No. I, first spermatocyte, anaphase.
71. No. I, second spermatocyte, metaphase.
72. No. I, second spermatocyte, anaphase.
73-74. No. II, first spermatocyte, metaphase.
75-77. No. III, first spermatocyte, metaphase.
78. No. III, first spermatocyte, anaphase.

Beach goldenrod aphid.

79 *a* and *b*. Parthenogenetic egg, polar body and prophase of first segmentation mitosis.
80-81. First spermatocyte, metaphase.
82. First spermatocyte, anaphase,
83. Second spermatocyte, metaphase.
84, Second spermatocyte, anaphase.

Tall goldenrod aphid.

85. First spermatocyte, before pairing of chromosomes.
86. First spermatocyte, prophase, showing paired chromosomes.
87. First spermatocyte, metaphase, Gilson.
88. First spermatocyte, early anaphase, Gilson.
89. First spermatocyte, metaphase, Flemming.
90. First spermatocyte, early anaphase, Flemming.
91-92. Second spermatocyte, metaphase.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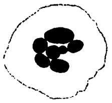
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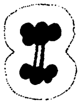
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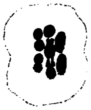
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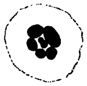
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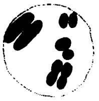
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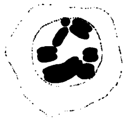
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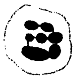
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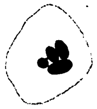
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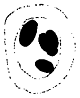
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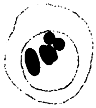
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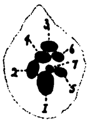

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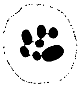
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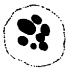
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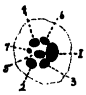

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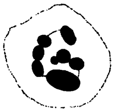
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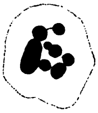
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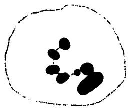
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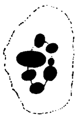
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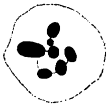
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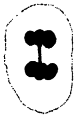
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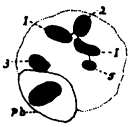

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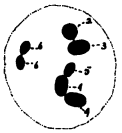

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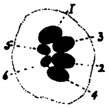

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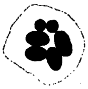
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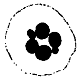
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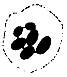
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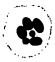
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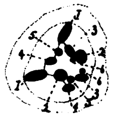

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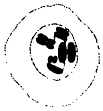
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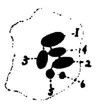

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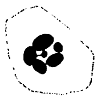
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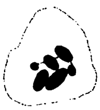
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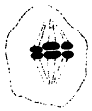
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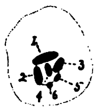

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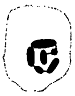
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PLATE IV.

Paper birch aphid.

FIG. 93. First spermatocyte, metaphase.

Clover aphid.

94. First spermatocyte, metaphase.
95-96. First spermatocyte, prophase.

Woolly beech aphid.

97-98. First spermatocyte, metaphase.
99. Second spermatocyte, metaphase.
100-101. First spermatocyte, anaphase.

Star cucumber aphid.

102-103. First spermatocyte, growth stage.
104. First spermatocyte, prophase.
105. First spermatocyte, metaphase, Hermann preparation.
106. First spermatocyte, metaphase, Gilson.
107. First spermatocyte, early anaphase.
108 *a* and *b*. Second spermatocyte, anaphase.
109. Second spermatocyte, anaphase.

Maple aphid.

110. First spermatocyte, metaphase.
111-112. First spermatocyte, anaphase.
113. First spermatocyte, prophase.

Pea aphid.

114. Parthenogenetic egg, metaphase of maturation spindle.
115-116. Parthenogenetic egg, 32-cell stage, metaphase of segmentation spindle.

Goumi aphid.

117-118. Winter egg, polar bodies.
119. Winter egg, male and female pronuclei.
120 *a* and *b*. Winter egg, prophase of segmentation mitosis.

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